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## STUDIES ON LIMULUS.

### I. *THE ENDOCRANIA OF LIMULUS, APUS, AND MYGALE,*

BY

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## INTRODUCTION.

IN 1888 I began a detailed study of the anatomy and development of *Limulus*, for the purpose of determining whether such a study would justify the conclusion that *Limulus* and other arachnids are closely related to ancestral vertebrates. That they are so related seemed probable in view of certain resemblances between the mode of development of the brain and eyes of *Limulus* and scorpion and those of vertebrates.

An elaborate attack on the problem was planned. The structure, development, and physiology of all the organs of *Limulus* were to be worked out, and a comparison made between them and the corresponding organs in vertebrates. But it was soon discovered that an inquiry of this nature, made from a special point of view, opens many side problems that necessitate frequent digressions in order to discuss tentative homologies

and other questions of a purely theoretical nature. This mode of treatment, therefore, is open to the constant danger of confusing fact and theory, of destroying the simplicity and directness of the purely descriptive parts, and of detracting somewhat from their permanent value. Moreover, comparisons instituted for the purpose of indicating broad homologies fail to carry conviction when standing alone, as they would have to do if each system of organs were treated separately. In such complicated problems as the origin of vertebrates, resemblances between several systems of organs and the corresponding parts in the hypothetical ancestors must be treated together, in order to show how one comparison harmonizes with and supports the other. It seemed best, therefore, to publish each descriptive part as soon as completed, with only an occasional attempt to point out the relations of certain organs in *Limulus* to the corresponding structures in vertebrates. At some later period we hope to combine our results into an organic whole.

While there may be differences of opinion as to the probable value of such an undertaking, there can be no doubt that if it leads to a fairly complete account of one species, that alone will be a sufficient return for the labor, and will give the work a value that cannot be derived from fragmentary accounts of different, even though closely related, forms. Moreover, this mode of treatment has obvious advantages, in that long familiarity with a given form enables the investigator to avoid the repetition of much preliminary work, and thus obtain his results more rapidly.

The senior author has for a long time regarded the endocranium, the branchial cartilages, and the segmental cartilages of the spinal cord of arachnids as forerunners of the corresponding cartilages in vertebrates, and he began to study their anatomy, histology, and development some ten years ago. A preliminary statement as to the structure of the endocranium in scorpions and *Limulus* was given in his paper on the "Origin of Vertebrates from Arachnids" (89). Since then, from time to time, many suggestive details have been gathered; but as no immediate opportunity was likely to present itself to get this material into proper shape for publication, it was decided to turn

over a part of it in the form of some rough sketches and notes to Mr. Redenbaugh, then a student in the biological laboratory at Dartmouth. Mr. Redenbaugh began the work anew, and with great care and skill has brought it to completion. His work on the endosternite of *Limulus* coincides very closely with my own. The drawings that accompany this paper have been made along the lines of my original sketches, some of which were intended to emphasize the relations of the cranial cartilages to the nervous system, and to show their resemblance to the corresponding parts in vertebrates. But every drawing has been made from the dissections, and with the utmost fidelity to details of size and position.

The descriptions of the endosternite of *Apus* and *Mygale*, with some unimportant exceptions, are the work of Mr. Redenbaugh.

Another paper, belonging to this series, on "The Structure and Development of the Nephridia, Branchial Cartilages, and Genital Ducts of *Limulus*," by Miss Hazen and myself, is completed and in the hands of the publishers, and further work on the histology and development of the endosternite and branchial cartilages is in progress.

The second paper of this series, that on "The Peripheral Nervous System of *Limulus*," was begun some years ago, and considerable progress was made on it. But the work was laid aside for a time, owing to the pressure of other duties; the notes and drawings were finally given to Mr. Redenbaugh, who went over the entire subject again, receiving from Dartmouth College, in recognition of his work, the degree of Ph.D.

A description of the "Structure and Development of the Central Nervous System of *Limulus*" is being prepared, also papers on the "Physiology of the Brain and Spinal Cord."

W. PATTEN.

The structure, which has been called by various names, *viz.*, prosomatic endochondrite, or endosternite, cartilaginous sternum, endocranium, and plastron, has been found in many of the *Arachnida* and in a few *Crustacea*. In all cases it is a



cartilaginous body of varied form lying dorsal to the cephalic ganglionic mass and ventral to the intestine. It functions as a centrum for the attachment of various muscles.

Schimkewitsch in '94 gave a very complete summary of all the literature upon the subject. Therefore it will only be necessary to call to mind those works which have an immediate bearing upon the forms herein described.

In '81 Lankester figured the plastrons of *Limulus*, *Mygale*, and *Scorpio*, and briefly compared them with each other in their general characteristics.

In '84 he gave a more complete account of the plastron of *Scorpio*. He figured and described those of *Apus*, *Mygale*, and *Limulus*, and gave something concerning the histology of all four of them.

In '85, with the aid of his pupils, Mr. Benham and Miss Beck, he compared the plastrons of *Limulus* and *Scorpio* with reference to their muscular attachments.

In '89 Patten figured and described the plastron of a different species of scorpion, and laid special stress upon the existence of the subneural arch, or "occipital ring." His figure differed from Lankester's principally in the absence of a diaphragm.

In '92, '93, and '94 came a series of papers by Schimkewitsch and Bernard, in which Bernard sought to homologize the apodemes of *Galeodes* with the endosternites of other arachnids, and to show that the endosternites of arachnids are apodematous structures due to fusion and compression of the cephalothoracic segments and later specialized for muscular attachments. He regarded the endosternite of *Limulus* as a derivation of the ventral muscle bands and believed it to be homologous with that of *Apus* and not with that of arachnids. Schimkewitsch, on the other hand, maintained that the apodemes of *Galeodes* were entirely represented by less developed apodemes in *Scorpio*, and that the endosternite was a morphologically different structure. He also stated that the arachnid endosternite was composed of two parts: (1) of a transversal muscle corresponding to the adductor muscle of *Crustacea*; and (2) of a pair, or perhaps several pairs, of mesodermic tendons connected with the transverse muscle strands.

## METHODS.

In the work upon *Limulus* both fresh and alcoholic material was employed, but in that upon *Mygale* and *Apus* only alcoholic specimens were used. The plastron of *Limulus*, with adjoining parts, was excised and allowed to soften in water until the muscles could be easily picked away. Dissection was then carried on under water or weak alcohol with fine pointed forceps. In other specimens the muscles were allowed to macerate completely, and were then brushed off, leaving the cartilage intact.

Serial sections of *Apus*, both transverse and longitudinal, were made, and a model of the plastron magnified seventy times was reconstructed from the longitudinal sections.

As *Mygale* material was scanty, only cross-sections of this animal were made. The viscera were cut from the cephalothorax and imbedded in celloidin. Sections were cut and mounted serially, stained in borax carmine, and a reconstruction of the cartilage made with an amplification of twenty times. With these models as guides, drawings were made and minute details added by careful study of the sections under the microscope.

## I. PLASTRON OF LIMULUS.

(See Pl. I, Figs. 1-4.)

1. *General Description.*—In the following descriptions, for reasons which will appear later, the terms “haemal” and “neural” will be substituted for dorsal and ventral, respectively, and the terminology of Lankester will be used in designating the parts of the endosternite.

In *Limulus* the plastron lies in about the center of the cephalothorax, with the anterior margin about opposite the chelicerae, and the posterior extremity on a level with the chilaria. The anterior processes extend some distance beyond the bases of the chelicerae. The mouth of the animal is located a little anterior to the center of the plastron, and from it the oesophagus passes forward between the anterior processes to form the <-shaped proventriculus, the haemal arm of which

communicates with the intestine. The intestine begins a short distance in front of the plastron and proceeds straight backward, close to the haemal side of the plastron. The oesophageal collar surrounds the entrance to the oesophagus, and lies entirely on the neural side of the plastron. The ventral cord passes posteriorly along the same side.

The plastron itself is roughly rectangular, with the long axis parallel to the long axis of the animal. It is produced anteriorly into two stout processes (anterior cornua, *a.c.*, Pl. I, Figs. 1 and 2), posteriorly into a cleft median process (posterior process, *p.pr.*, Figs. 1-4) and a pair of short bars (capsuliginous bars, *cap.b.*, Figs. 1-4).

Laterally, near the anterior margin, are two pairs of long rod-like tendons (lateral cornua, *l.c.*, Figs. 1 and 2), and near the posterior border is a pair of short stout processes (latero-posterior processes, *l.p.pr.*, Figs. 1-4).

Neurally a pair of processes arise from near the bases of the latero-posterior processes, pass around the ventral cord, and unite on the neural side of it to form the occipital ring (*oc.r.*, Figs. 1, 3, and 4). This ring is connected posteriorly with the capsuliginous bars (*cap.b.*).

Haemally a pair of short stout processes (dorsal or haemal processes, *h.pr.*, Fig. 2) arise midway along the lateral margins of the plastron.

The body of the plastron is a thick plate of fibroid cartilage, flat or slightly concave on the neural side, with the lateral margins (*m.r.*, Figs. 1 and 3) sharply elevated, particularly towards the anterior end. The haemal side is concave in transverse section. The lateral edges are much thickened anteriorly, and produced beyond the body of the plastron to form the anterior cornua.

2. *The anterior cornua (a.c., Figs. 1 and 2)* are a pair of stout transversely flattened processes. To each process are attached three muscles, which go to the haemal side of the carapace; one directly forward from the extremity, one perpendicularly from the inner surface of the process, and one obliquely forward from the haemal margin.

The neural margins of the processes and the entire lateral



portions of the plastron, including the latero-posterior processes, give attachment to the plastro-coxal muscles of the thoracic appendages from the second to the sixth pairs.

Anteriorly the muscles do not cover the neural surface of the plastron, but posteriorly, as the muscles increase in size with the increase in size of the appendages, they encroach upon the neural surface even to the median line. There is, therefore, on the anterior neural surface of the plastron a triangular space which, except for a few loose strands (plastro-buccal muscles) going to the oesophagus, is free from muscles and comparatively smooth.

3. *The lateral cornua* (*l.c.*, Figs. 1 and 2) spring from the anterior haemal side of the plastron. They are long and slender, and each is attached by a short muscle to the haemal side of the carapace, close to the origins of the tergo-coxal muscles. The first pair of cornua pass between the tergo-coxal muscles of the third and fourth pairs of appendages, the second pair between the tergo-coxals of the fourth and fifth pairs of appendages.

The bases of these lateral cornua give to the plastron a greater thickness at the anterior border than in the middle.

4. *The haemal processes* (*h.pr.*, Fig. 2) are very stout, and spring from the haemal side near the lateral edge of the plastron, about halfway between the latero-posterior processes (*l.p.pr.*) and the lateral cornua (*l.c.*). Their bases anteriorly are close to the edge of the plastron, but posteriorly, owing to the widening of the plastron towards the latero-posterior processes, they lie about halfway between the middle and the edges of the plastron. These processes incline slightly outwards, and each gives attachment to two muscles: one going haemally and a little laterally from the extremity of the process to the carapace, and the other posteriorly and slightly haemally from the posterior margin of the process to the first entapophysis.

5. *The latero-posterior processes* (*l.p.pr.*, Figs. 1-4) are lateral expansions of the posterior portion of the plastron. They are flattened haemo-neurally, and rapidly taper to a point. Along the posterior margin of each, on the neural side, is a sharp ridge, which towards the median line is continuous with the base of the occipital ring. The latero-posterior processes give attach-

ment to some of the plastro-coxal muscles of the sixth pair of legs, which are the most powerful appendages of the animal.

A marginal ridge is formed (*m.r.*, Fig. 1) on the neural side of the plastron, which becomes quite prominent anteriorly; posteriorly it dwindles to a low rounded shoulder at the base of the latero-posterior process, then rises a little, to form the base of the occipital ring (*oc.r.*, Figs. 1, 3, and 4). There is also a shoulder (*s.*, Fig. 4) running across the body of the plastron, connecting the posterior borders of the latero-posterior processes.

6. *The posterior process (p.pr.*, Figs. 1-4) begins as a thickening on the haemal side of the plastron between the haemal processes. It increases in thickness posteriorly, and ends in a bifid process, each division of which is deeply grooved on the haemal side. Along the whole haemal side of the process are attached two large muscles going to the first pair of entapophyses.

On the body of the plastron, on both sides of the base of the posterior process, are attached longitudinal abdominal muscles, passing backward between the last described muscles and the plastron. The attachments of these muscles extend a little anterior to the haemal processes. In front of this the body of the plastron is destitute of muscles on the haemal side.

A pair of small chilial muscles are attached to the haemal side of the extremity of the posterior process.

7. *The capsuliginous bars (cap.b.*, Figs. 1-4) are a pair of processes of peculiar structure arising from the posterior margin of the plastron, alongside the posterior process. The latero-posterior process on each side is extended backwards as a much thinner layer of cartilaginous tissue. It is flush with the neural surface of the posterior process, but where it joins the latero-posterior process there is a very abrupt shoulder upon the neural side and a slight one upon the haemal side. To the posterior margins of these thin portions are attached the rod-like bars. The latter bend neurally and slightly toward the median line, enter the bases of the chilaria, and are attached to their posterior sides.

A small transverse muscle joins the distal ends of the two bars. Two other small muscles run to the chilaria from the thin portions of the plastron near the bases of the bars.













It is especially noteworthy that the capsuliginous bars differ histologically from the rest of the plastron, and that they are united with the thinner portions of the plastron by what appears to be a true joint. The body of the plastron is composed of fibroid cartilage, while the bars are of capsuliginous cartilage, exactly like that found in the abdominal appendages, the histology of which has been described by Gegenbaur ('58) and Lankester ('84) and more recently by Gaskell ('97).

8. *The Occipital Ring* (*oc.r.*, Figs. 1, 3, and 4). — At the points where the marginal ridges (*m.r.*, Fig. 1) meet the bases of the latero-posterior processes, two outgrowths are formed which are united with each other distally on the neural side of the ventral cord by a connective tissue membrane. At their bases the processes are slender, but distally they enlarge and thicken, and are joined to the capsuliginous bars (*cap.b.*) by strands of connective tissue.

That the *occipital ring* or subneural arch thus formed is a true cartilaginous ring is beyond doubt, for serial sections of a young animal through this region show the continuity of the cartilaginous tissue entirely around the cord (Fig. 4). Upon the neural side of the ring are two depressions (*ch.m.*, Figs. 1, 3, and 4), to which are attached a pair of muscles going to the insides of the chilaria. From the anterior side of the ring, muscle strands pass forward to the integument immediately behind the mouth.

9. *Accessory Structures.* — Besides the processes and muscle attachments above mentioned, there are found along the lateral edges of the plastron on the haemal side, and attached to it by connective tissue fibers, tough membranes (*mem.*, Fig. 2), to which are attached the "veno-pericardiac muscles" of Lankester, or the "brides transparentes" of Milne-Edwards (*v.p.m.*<sup>1-2</sup>, Fig. 2). Anteriorly this membrane springs from the side of the plastron just back of the lateral cornua. About midway between the lateral cornua and the haemal process it affords attachment to the anterior or first veno-pericardiac muscle (*v.p.m.*<sup>1</sup>, Fig. 2). Just anterior to the latero-posterior process it gives attachment to the second veno-pericardiac muscle (*v.p.m.*<sup>2</sup>, Fig. 2), and at the same point sends a bundle of con-

densed connective tissue neurally to the integument between the fifth and sixth pairs of legs. A similar bundle unites it with the integument just outside of the chilaria, and from here on it is attached by a double base all along the integument of the abdomen, between the ventral longitudinal and the "branchio-thoracic" muscles. It furnishes attachment for all the veno-pericardiac muscles.

Benham, in his paper "On the Muscular and Endoskeletal Systems of *Limulus*" ('85), has described the veno-pericardiac muscles as attached to the dorsal side of the longitudinal venous sinus. The venous sinuses of *Limulus*, as a rule, have no walls substantial enough for a muscle attachment; but posterior to the second veno-pericardiac muscle the above-described membrane is double and spans the venous sinus. Anterior to this point the venous sinuses have no connection with the membrane.

10. *Foramina*.—Two pairs of foramina for the passage of nerves have been found. One pair (*f*.<sup>1</sup>, Figs. 1–3) may be seen at the bases of the latero-posterior processes, just outside the neural marginal ridge and appearing on the haemal side of the plastron, a little posterior to the haemal processes. These two foramina furnish passage for the intestinal branches (*in.n*.<sup>6</sup>, Fig. 3) of the haemal nerves belonging to the sixth thoracic neuromere.

The second pair of foramina (*f*.<sup>2</sup>, Figs. 1–3) are located in the posterior thinner portion of the plastron, near the bases of the processes which form the occipital ring. Through these pass the intestinal branches (*in.n*.<sup>7</sup>, Fig. 3) of the haemal nerves of the chilarial neuromere.

11. *Relation of the Brain to the Plastron*.—Following the nomenclature adopted by Patten ('89, '93), the term "brain" will be applied to the entire circumoesophageal collar; the term "fore-brain" to the supraoesophageal portion, or the part derived from the preoral lobes of the embryo; "hind-brain" to the part formed by the fusion of the six thoracic neuromeres; and "accessory brain" to that portion formed from the two abdominal neuromeres which fuse secondarily with the thoracic neuromeres.

The brain forms a close-fitting collar around the oesophagus and lies a little in front of the center of the plastron. Six pairs of large pedal nerves radiate from the neural side of the collar and innervate the six pairs of thoracic appendages. Six pairs of integumentary nerves belonging to the same neuromeres radiate from the haemal side and innervate the skin and other organs on the haemal and neural sides of the carapace.

In Pl. I, Fig. 3, only the posterior half of the collar is represented; *n.n.4*, *n.n.5*, and *n.n.6* are the three posterior pedal or neural nerves of the thoracic neuromeres; *h.n.4*, *h.n.5*, and *h.n.6* are the three integumentary or haemal nerves of the same neuromeres. Of these, the haemal nerves (*h.n.6*), which belong to the same metamere as the sixth pair of legs, give off small branches (*in.n.6*) which pass through foramina (*f.1*) in the plastron and communicate with a sympathetic system supplying the intestine and the longitudinal abdominal muscles. The main portions of the nerves give off branches to the region of the heart, and then ramify over the skin of the haemal and neural surfaces of the cephalothorax.

The ventral cord (*v.c.*), the chilial (*n.n.7*), and the opercular (*n.n.8*) nerves, and two pairs of integumentary nerves (*h.n.7* and *h.n.8*) belonging to the chilial and opercular neuromeres, pass out from the posterior side of the brain through the occipital ring.

The chilial nerves (*n.n.7*, Figs. 3 and 4) arise on the neural side of the posterior end of the oesophageal collar and pass directly backwards through the occipital ring close to its neural portion and innervate the chilaria.

On the haemal side of the collar arise a pair of integumentary or haemal nerves (*h.n.7*, Figs. 3 and 4) belonging to the chilial neuromere. They diverge laterally and pass through the occipital ring, sending branches (*in.n.7*) through foramina (*f.2*) to supply the intestine and the longitudinal abdominal muscles. The main portions of the nerves bend sharply outwards after leaving the occipital ring, and, after giving off cardiac branches, supply the skin on the haemal and neural surfaces of the anterior portion of the abdomen. The chilaria, though apparently

in the thoracic region, belong primarily to the category of abdominal appendages, as shown (1) by the development of the chilial neuromere; (2) by the distribution of the haemal nerves of the neuromere; and (3) by the possession of a gill bar of capsuliginous cartilage closely resembling those found in the other abdominal appendages.

The opercular nerves (*n.n.*<sup>8</sup>, Figs. 3 and 4) arise a little posterior to the chilial nerves and pass backward alongside the ventral cord through the occipital ring and innervate the genital operculum. The haemal nerves (*h.n.*<sup>8</sup>, Figs. 3 and 4) belonging to this neuromere arise slightly posterior to the haemal nerves of the chilial neuromere, and, diverging at a less angle than the preceding, pass through the occipital ring and out towards the sides of the body immediately posterior to the capsuliginous bars (*cap.b.*). As they turn outwards each sends a branch (*in.n.*<sup>8</sup>, Figs. 3 and 4) haemally to the intestine and longitudinal abdominal muscles. The main part of the nerve finally sends a branch to the heart, and then distributes itself over the surface of the abdomen. The ventral cord (*v.c.*, Figs. 3 and 4) passes straight back from the oesophageal collar through the occipital ring and does not branch until it reaches the ganglion of the first gill metamere.

## II. ABDOMINAL ENDOCHONDRITES OF *LIMULUS*.

In the abdominal region of *Limulus* is a series of cartilages spanning the ventral cord on the neural side. There are six in all, one at the base of each of the abdominal appendages, from the operculum to the fifth gill. Like the plastron, they are composed of fibroid cartilage and serve as centra for the attachment of muscles, but they differ from the plastron in being placed on the neural side of the central nervous system instead of upon the haemal side. These cartilages vary considerably in different individuals and in different metameres of the same individual, but the one represented in Fig. 3 (*ab.en.*) is typical. It consists of an irregularly shaped body with a flat neural surface, which is in contact with the integument except across the middle portion. This middle part lies directly under



the base of the operculum and is indented by two pits (*op.m.*), which represent the origin of a pair of muscles inserted on the inside of the appendage.

The posterior and anterior prolongations of the endochondrite (*p.p.* and *a.p.*, Fig. 3) serve partly for the attachment of muscle strands of the longitudinal muscles of the abdomen, but in many cases the anterior and posterior processes of successive endochondrites are continuous with each other. On the haemal side of the endochondrite is a pair of haemal processes (*h.p.*, Fig. 3), one on each side of the ventral cord. These project haemally, and a little outward and backward, and furnish attachment for a pair of haemo-neural muscles inserted on the haemal side of the carapace just median to the entapophyses.

### III. THE ENDOSTERNITE OF APUS.

(Pl. II, Figs. 5-10.)

This structure, like that of *Limulus*, is located in the cephalothorax, between the central nervous system and the intestine. It lies behind the mouth and opposite the mandibles. The body of the plastron is elongated in a direction transverse to the long axis of the animal, and its flaring ends (*m.*, Figs. 5, 6, and 10) give attachment to the powerful adductor muscles of the mandibles.

On the posterior side a pair of chitinous apodemes (*apo.*, Figs. 5, 6, 8-10) project into the plastron. These are formed by the invagination of the chitin between the bases of the first and second appendages behind the mandibles. These appendages have been called the first and second maxillae.<sup>1</sup> Lankester regards them as one appendage consisting of two parts and calls it a maxilla. Bernard calls the anterior portion a cleft underlip, and the posterior portion the first maxilla.

From the inner ends of the apodemes a pair of tendonous cords run forward directly through the body of the plastron at right angles to its fibers, and emerge on the anterior side as a pair of anterior cornua (*a.c.*, Figs. 5, 6, 8, and 9). These proc-

<sup>1</sup> A. Gerstaecker, *Die Klassen und Ordnungen der Arthropoden*, Bd. v. Crustacea.

esses are each split into three divisions. The neural portion (*n.*, Figs. 5, 6, and 9) is in contact with the integument at the side of the mouth, and furnishes attachment for a few muscle strands going to the integument anterior to the labrum. The middle (*m.*, Figs. 5, 6, and 9) and haemal divisions (*h.*, Figs. 5-9) both furnish attachment for muscles going to the posterior side of the flexure of the oesophagus. The haemal division also continues as a thin strand around the side of the oesophagus to the haemal side of the carapace, anterior to the eyes. The neural and middle divisions are rather stout, while the haemal one is thin and joined at the base to its fellow upon the opposite side. Thus in median longitudinal section the plastron appears to terminate in a knife edge (*h.*, Fig. 7).

Posteriorly the plastron terminates, in the median portion, in a thin membrane (*z.*, Figs. 5-8), which runs backward and is attached to the integument between the longitudinal commissures of the ventral cord. It is continued laterally onto the posterior sides of the apodemes (*z.*, Fig. 8).

The ventral longitudinal muscles of the abdomen are attached to the posterior sides of the apodemes and to the plastron itself on each side of the median line. A process (*x*, Figs. 5 and 8) projecting neurally from each apodeme sends muscle strands to the inside of the second pair of maxillae.

Haemo-neural muscles are attached to the haemal sides of the apodemes. There are also a pair of muscles inserted on the posterior neural portion of the plastron (*y.*, Figs. 5, 7, and 10), passing to the integument between the longitudinal commissures, just back of the first cross-commissures.

#### IV. ENDOSTERNITE OF MYGALE.

(Pl. II, Figs. 11 and 12.)

This plastron, like those heretofore described, lies between the alimentary canal and the central nervous system. Its general contour from the neural aspect is oval, with the longer axis parallel to the long axis of the animal.

The anterior edge is deeply indented by a bay running to the

middle of the plastron, thus forming a pair of very large anterior cornua (*a.c.*, Figs. 11 and 12).

The body of the plastron may be considered as a plate of cartilage with crenate margins and concave on the neural side. Radiating from a common center on the neural side are four paired, plate-shaped processes (*n.pl.*<sup>1-4</sup>, Fig. 11), and one posterior unpaired one (*n.pl.*<sup>5</sup>, Fig. 11). The posterior one is thinner than the others and gradually tapers out, ending in three low ridges. The anterior pair borders the inner neural margins of the anterior cornua (*a.c.*). From about the middle of the anterior cornua spring a pair of processes (*n.pr.*, Figs. 11 and 12), which bend around the brain and attach themselves to the integument close together on the neural side.

On the haemal side of the plastron two high ridges converge from the distal ends of the anterior cornua to the posterior end of the plastron, forming a deep gully between them, in which lies the alimentary tract. These ridges are split up into five paired haemal processes (*h.pr.*<sup>1-5</sup>, Fig. 12) of unequal length, those in the middle being longer than those at either end. The plastron ends posteriorly in a short median process (*p.pr.*, Figs. 11 and 12).

The muscles arising from the plastron are too numerous and complicated to allow of a full description in this paper. From nearly the whole of the neural side muscles go to the legs; haemo-neural muscles are attached to the haemal processes (*h.pr.*<sup>1-5</sup>), and longitudinal muscles to the posterior process (*p.pr.*).

The brain lies just haemal to the neural processes (*n.pr.*), which are in contact with the integument. The oesophagus passes through the brain and between the anterior cornua to the sucking stomach, which lies in the groove on the haemal side of the plastron. Muscle strands run from the stomach to the walls of the groove.

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There are, therefore, four distinct structures in arachnids that may serve as parts of a true endoskeleton :

I. The *endosternite* or *endocranium*, a broad flat plate of fibro-cartilage lying on the dorsal side of the brain, and serving

mainly for the attachment of the muscles that move the thoracic appendages and the flexor muscles of the thorax and abdomen. In scorpions and *Limulus* a complete cartilaginous occipital ring is formed about the spinal cord, near its union with the brain. The plate is a continuous structure, and there is no indication whatever that it consists of two separate lateral bars, as maintained by Gaskell.

We recognize three parts to the endosternite of arachnids, namely, two lateral bars, a broad plate of cartilage on the dorsal side of the nervous system uniting the posterior ends of the bars, and a bridge of cartilage on the ventral side, which, together with the above-mentioned parts, forms a continuous ring about the anterior end of the spinal cord.

This whole structure, after the extensive reduction of the thoracic appendages and the muscles that move them, is retained, apparently, in the ancestral vertebrates, and becomes, as was first pointed out by Patten in '89, the primordial cranium. The lateral bars, the transverse plate, and the ventral arch correspond respectively to the trabeculae, the basilar plate, or parachordals, and the occipital ring. If we add the olfactory and auditory capsules and roof over the remaining dorsal surface, we obtain a complete cartilaginous cranium like that of higher forms. As we shall show elsewhere (*American Naturalist*), Gaskell's attempt to utilize the endosternite in his version of the origin of vertebrates is a complete failure, since under the conditions of his theory it must be torn apart and put together again upside down, with the old occipital ring replaced by a new one on the opposite side of the endosternite from the one on which it actually occurs. The endosternite has a true cartilaginous consistency, and is composed of a mass of interwoven fibers containing variously formed stellate lacunae, which may be very clearly seen after macerating out the cell contents. The lacunae are united by numerous anastomosing canaliculi, which in the living cartilage contain minute branching processes of the cartilage cells situated in the lacunae.

II. The six segmentally arranged *cartilages* of the *spinal cord* (Pl. VI, Fig. 1) contain the same kind of cartilage as the endosternite. They are the forerunners, we believe, of the



segmental cartilages found in corresponding regions in primitive vertebrates, and which give rise to the vertebral column. The cartilages serve evidently as points of attachment for segmentally arranged muscles that meet at those points. But we must not conclude that that is the only reason why the cartilages are there, because cartilages are not always formed at points where muscles are attached to one another. It is obviously impossible to carry the comparison with vertebrate cartilages any farther. It is enough for our purpose to show that the conditions in *Limulus* are such as to produce a series of segmentally arranged cartilages about the spinal cord, similar to those in primitive vertebrates.

III. There are seven pairs of *branchial cartilages* composed of an entirely different tissue histologically (and chemically, also, according to Gaskell) from that in the endosternite and the segmental cartilages.

The first pair arise from the inner surface of the chilaria, and are attached to the posterior margin of the endocranium so that they appear to form a part of it. The remaining six pairs arise from the base of the abdominal appendages and go to the corresponding entopophyses. They develop, as will be shown in one of the papers of this series, at a very early embryonic period as clearly defined outgrowths of the walls of the mesoblastic somites. Their union with the epidermis is secondary, and they are in no wise derived from the modification of chitinous ingrowths from the epidermis, as maintained by Gaskell.

These branchial bars of the mesothorax correspond, we believe, to the cartilaginous bars of the visceral clefts of vertebrates, as we first indicated in '89. In '93 we called attention to the surprising histological resemblance between these cartilages and those of *Petromyzon*, and still later ('96) we showed that there was a certain number of embryos in which one or more pairs of appendages were invaginated instead of evaginated. Transverse slits were thus formed along the sides of the head, resembling vertebrate gill slits and recalling to mind the lung books of scorpions and spiders. In *Limulus* the appendages most frequently invaginated, the thoracic ones, are not provided with gills. This again is suggestive since in vertebrates

the most anterior visceral arches are likewise devoid of gill lamellae. On the neural side of each thoracic appendage of *Limulus*, except the first and last, is a large group of sense organs supplied by a special ganglionated tegumentary nerve, *i.e.*, the gustatory organs of the coxal spines (Patten, '93) and the sense organs on the endopodite of the abdominal appendages. These sense organs and nerves correspond in position and in mode of development respectively to the suprabranchial sense organs and the rami dorsali of the cranial nerves of vertebrates.

IV. *The Dermal Skeleton.*—*Limulus* is the only invertebrate where the chitinous exoskeleton has begun to form a system of true dermal bones (Patten, '94). They arise as innumerable ingrowths of the ectoderm that unite to form a mass of anastomosing chitinous trabeculae. The tissue thus formed resembles coarse cancellated bone, but more especially the coarse bony networks that form the inner layers of the cephalic shields of the *Cephalaspidæ*. Within these trabeculae are numerous cavities, which, after the shell is macerated and dried, become filled with air, and then bear a strong resemblance to true bone lacunae. They are spindle-shaped lacunae, with two or more very fine canaliculi leading off from them, which appear to unite in some cases with the canaliculi of neighboring lacunae. In living tissue the lacunae are filled with a substance resembling protoplasm, and the larger ones appear to be nucleated. Thus, an entirely new dermal structure is forming here unlike that known in any other invertebrate; namely, local ingrowths of the ectoderm, forming a network of chitinous trabeculae, into which numerous cells migrate to form true bone corpuscles.

As the trabeculae cannot be shed periodically, like the rest of the exoskeleton, they are retained permanently within the body. Since, as we now know, some forms of chitin are very closely allied to chondrin, perhaps this condition may be the means of ultimately completing the chemical metamorphosis of chitin into chondrin. But there is no evidence that other chitinous ingrowths, such as the entopophyses or the apodemes of many arthropods, are invaded by cells, or that they have

made any decided approach in chemical composition towards true bony or cartilaginous structures.

We can readily understand how such a sub-dermal framework as we see in *Limulus* might lose its connection with the epidermis, and the latter form a continuous layer above it. Then the hard superficial chitinous armor would no longer be needed, either for the attachment of muscles or for protection, and might disappear altogether, thus obviating the dangers of periodically shedding the old chitinous exoskeleton.

Thus *Limulus* appears to have laid the foundation for an elaborate system of internal supports; namely, the primordial cranium, the branchial cartilages, the cartilages of the spinal cord, and the sub-ectodermal structures which resemble dermal bones. These parts have different modes of origin, and differ widely histologically from one another, yet they agree in all essential features with the corresponding parts of the vertebrate skeleton. In vertebrates we so frequently see these structures welded together into a common framework for the whole body, that we underestimate the importance of certain facts of vertebrate ontogeny, which indicate more and more clearly that the vertebrate skeleton is also composed of several parts quite different in structure and origin.

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## REFERENCE LETTERS.

<i>ab. en.</i>	abdominal endochondrite.	<i>mem.</i>	membrane to which are attached the veno-pericardiac muscles.
<i>a. c.</i>	anterior cornua.	<i>m. r.</i>	marginal ridge.
<i>a. p.</i>	anterior process of abdominal endochondrite.	<i>n.</i>	neural division of anterior cornu of plastron of Apus.
<i>apo.</i>	apodeme.	<i>n. n.<sup>4-8</sup></i>	neural nerves.
<i>cap. b.</i>	capsuliginous bars.	<i>n. pl.</i>	neural plates.
<i>ch.</i>	chilaria.	<i>n. pr.</i>	neural processes.
<i>ch. m.</i>	origin of chilarial muscles.	<i>oc. r.</i>	occipital ring.
<i>cr. com.</i>	cross-commissures.	<i>oe. col.</i>	oesophageal collar.
<i>f.<sup>1-2</sup></i>	foramina.	<i>op. m.</i>	origin of opercular muscles.
<i>h.</i>	haemal division of anterior cornu of plastron of Apus.	<i>p. p.</i>	posterior processes of abdominal endochondrite.
<i>h. n.<sup>4-8</sup></i>	haemal nerves.	<i>p. pr.</i>	posterior process.
<i>h. p.</i>	haemal processes of abdominal endochondrite.	<i>s.</i>	shoulder joining latero-posterior processes.
<i>h. pr.</i>	haemal processes of plastron.	<i>v. p. m.<sup>1-2</sup></i>	veno-pericardiac muscles.
<i>in. n.<sup>6-8</sup></i>	intestinal nerves.	<i>w., x., &amp; y.</i>	points of muscular attachment on plastron of Apus.
<i>int.</i>	integument.	<i>z.</i>	membrane attached to posterior side of plastron of Apus.
<i>l. c.</i>	lateral cornua.		
<i>l. p. pr.</i>	latero-posterior processes.		
<i>m.</i>	middle division of anterior cornu of plastron of Apus.		



## EXPLANATION OF PLATE I.

FIG. 1.  $\times 2$ . Endosternite of *Limulus* from neural side, anterior extremity towards top of plate. Only the bases of the lateral cornua (*l. c.*) are represented. The anterior cornua (*a. c.*) are prolongations of the thickened sides of the plastron. The sides of the plastron are roughened by muscular attachment. Inside the marginal ridge (*m. r.*) the floor of the plastron anteriorly is smooth and marked only by the slight transverse furrows indicating the course of the fibers of the cartilage, for there are no muscles attached here except a few strands going to the oesophagus. Posterior to this space the irregular markings indicate an area of muscular attachment, the anterior limit of which is marked by a V-shaped outline. The marginal ridge (*m. r.*) runs along each side of the plastron, from the anterior cornua to the occipital ring (*oc. r.*). Anteriorly the ridge rises sharply above the floor of the plastron, but posteriorly it is only a low, rounded shoulder.

The occipital ring (*oc. r.*), shaded lighter than the rest of the plastron, is marked on the anterior margin by the attachments of muscle fibers going to the integument behind the mouth. On the top are two shallow pits (*ch. m.*) in which a pair of muscles going to the chilaria take their origin. From the posterior side of the ring thin strands of connective tissue go to the capsuliginous bars (*cap. b.*). The capsuliginous bars (*cap. b.*) arise from the thinner portion of the plastron, in the angle between the posterior process (*p. pr.*) and the latero-posterior processes (*l. p. pr.*). The posterior process (*p. pr.*) is unpaired and is divided into two divisions.

The first pair of foramina (*f.<sup>1</sup>*) are represented by black dots at the bases of the latero-posterior processes in the thick portion of the cartilage; the second pair (*f.<sup>2</sup>*) by the light circles in the thin portion back of the occipital ring.

FIG. 2.  $\times 2$ . Endosternite of *Limulus* from haemal side. The distal ends of the lateral cornua upon the left side are disconnected from the proximal portions. These cornua are fully one-third longer than here represented. The drawing shows their mode of attachment to the haemal side of the plastron and the courses of the fibers in and about their bases. This portion of the haemal surface is free from muscles. The area of muscular attachment begins with the splintery appearance just anterior to the haemal processes (*h. pr.*). The edges of the plastron between the lateral cornua and the haemal processes are slightly elevated. The haemal processes (*h. pr.*) are stout and rise a considerable distance above the body of the plastron.

The first pair of foramina (*f.<sup>1</sup>*) appear as dark slits a short distance behind the haemal processes.

The posterior process (*p. pr.*) begins as a low ridge in the median line opposite the haemal processes. Each division of its forked end is deeply grooved. The capsuliginous bar (*cap. b.*) on the left is shown entering the base of one of the chilaria (*ch.*), to the posterior side of which it is attached. Running along the left side of the plastron is a connective tissue membrane (*mem.*) to which the venopericardiac muscles are attached. The bases of the two anterior of these muscles (*v. p. m.<sup>1-2</sup>*) are represented. Neural to the base of the second muscle (*v. p. m.<sup>2</sup>*) the membrane is attached to the integument (*int.*) by a bundle of connective tissue

fibers. Alongside the chilaria it is attached to the integument by a similar bundle of connective tissue.

FIG. 3.  $\times 2\frac{1}{2}$ . Posterior portion of plastron of *Limulus* and first abdominal endochondrite, with posterior half of oesophageal collar (*oe. col.*) and nerves, neural side. Four of the cross-commissures (*cr. com.*) are shown. In each neuromere are two pairs of nerves—a neural pair (*n. n.<sup>4-8</sup>*) and a haemal pair (*h. n.<sup>4-8</sup>*). Of the neural nerves, *n. n.<sup>4</sup>*, *n. n.<sup>5</sup>*, and *n. n.<sup>6</sup>* supply the fourth, fifth, and sixth pairs of legs respectively; *n. n.<sup>7</sup>*, the chilaria; and *n. n.<sup>8</sup>*, the operculum. All the haemal nerves supply the skin of the carapace and other organs; *h. n.<sup>6</sup>* and *h. n.<sup>7</sup>* give off small nerves (*in. n.<sup>6</sup>* and *in. n.<sup>7</sup>*) through the first and second pairs of foramina (*f.<sup>1</sup>* and *f.<sup>2</sup>*) respectively; *h. n.<sup>8</sup>* also gives off a small nerve (*in. n.<sup>8</sup>*) just posterior to the plastron. This is seen through the semi-transparent connective tissue attached to the capsuliginous bars. These small nerves (*in. n.<sup>6-8</sup>*) communicate with the sympathetic system supplying the intestine and longitudinal abdominal muscles. The nerves *n. n.<sup>7-8</sup>*, *h. n.<sup>7-8</sup>*, and the ventral cord (*v. c.*) pass through the occipital ring. The ventral cord passes haemal to the abdominal endochondrites, the first of which (*ab. en.*) lies at the base of the operculum. The shallow pits (*op. m.*) on the surface of this endochondrite, similar to those on the occipital ring, represent the attachments of a pair of muscles entering the operculum. Posterior and anterior to these pits the endochondrite is in contact with the integument. In some cases the anterior and posterior processes (*a. p.* and *p. p.*) of successive endochondrites are continuous with each other as thin strands of connective tissue. The haemal processes (*h. p.*) which straddle the ventral cord give attachment to haemo-neural muscles.

FIG. 4.  $\times 2$ . Endosternite of *Limulus* from posterior side, showing the occipital ring (*oc. r.*) and its relation to the nerves. The portion of the plastron anterior to the occipital ring is not represented.

*S.* is a thickened shoulder uniting the bases of the latero-posterior processes (*l. p. pr.*). Other reference letters as in Fig. 3.









## EXPLANATION OF PLATE II.

FIG. 5.  $\times 45$ . Endosternite of *Apus*, neural side, anterior extremity towards top of plate. The jagged ends (*m.*) furnish attachment for the muscles of the mandibles. The anterior cornua (*a. c.*) are each divided into three parts (*h.*, *m.*, and *n.*). A pair of chitinous apodemes (*apo.*) project into the endosternite on the posterior side. Muscles going to the maxillae are attached to the small processes at *x*. A few strands going to the integument are attached at *y*. The process *z* is a thin membrane attached to the integument between the longitudinal commissures of the ventral cord.

FIG. 6.  $\times 45$ . Endosternite of *Apus*, haemal side. Longitudinal abdominal muscles are attached to the jagged processes (*w.*) on the posterior side. Other reference letters as in Fig. 5.

FIG. 7.  $\times 60$ . Sagittal section of endosternite of *Apus* near median line; anterior end towards the right of figure. It shows the neighboring integument (*int.*) with the muscles going to it from *y*, the relations of the membrane *z* to the integument, and the positions of the cross-commissures (*cr. com.*<sup>1-3</sup>) with reference to the endosternite.

FIG. 8.  $\times 60$ . A section of the plastron of *Apus* a little farther from the median line than the preceding. It shows the apodeme (*apo.*) and the tendinous cord running from it, through the plastron, to the anterior cornu (*a. c.*). The process *x*, the membrane *z*, and the projections due to attachment of abdominal muscles to posterior side of apodeme are cut by this section.

FIG. 9.  $\times 60$ . A section of the plastron of *Apus* still farther from the median line. The apodeme (*apo.*) is approaching the exterior. All three divisions (*h.*, *m.*, and *n.*) of the anterior cornu (*a. c.*) are cut through.

FIG. 10.  $\times 60$ . A cross-section through posterior part of plastron of *Apus*. It cuts the inner ends of the apodemes (*apo.*), the muscles at *y*, and a pair of ganglia of the ventral cord (*v. c.*).

FIG. 11.  $\times 10$ . Endosternite of *Mygale*, neural side. The anterior cornua (*a. c.*) are very large. The neural processes (*n. pr.*) are in contact with the integument. The neural plates (*n. p.*<sup>1-5</sup>) project vertically from the body of the plastron. The ends of the five haemal processes (*h. pr.*<sup>1-5</sup>) protrude beyond the edges of the plastron. The posterior process (*p. pr.*) is not cleft, as in *Limulus*.

FIG. 12.  $\times 10$ . Endosternite of *Mygale*, haemal side. Five pairs of haemal processes (*h. pr.*<sup>1-5</sup>) rise from the sides of the hollow in which the intestine lies. Reference letters as in Fig. 11.











# A CONTRIBUTION ON THE MINUTE ANATOMY OF THE SYMPATHETIC GANGLIA OF THE DIF- FERENT CLASSES OF VERTEBRATES.

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## INTRODUCTION.

EVEN a cursory review of our knowledge of the minute anatomy of any tissue or organ teaches us that the various steps in the perfection of our knowledge are in a large measure synchronous with advances made in microscopical technic. This is, perhaps, to no one more clearly shown than to him engaged in neurological work. The introduction of solutions of osmic acid and gold chloride, the Weigert's haematoxylin method, the methods of Golgi and Ehrlich, and the numerous other special methods, have, each in its turn, directed the attention of investigators to results hitherto unattained. This is especially true of the chrome silver and the methylene blue method; the results obtainable by these two methods, variously modified (embracing, as they do, a large portion of our more exact knowledge of the shape of neurons, and especially of their relation to each other and to the myriads of cells under their influence), have led many workers to devote much of their time to the investigation of various portions of the central and peripheral nervous system of vertebrates, and to some extent, also, of the still larger group of invertebrates. Of this number many, no doubt, encouraged by the earlier results of Kölliker and Ehrlich, have used these methods for the investigation of the sympathetic or ganglionic nervous system. And it is gratifying to reflect that the researches of Kölliker, Ehrlich, Aronson, Arnstein, Smirnow, Cajal, Van Gehuchten, V. Lenhössék, Sala, Retzius, d'Erchia, Dogiel, and others have very much broadened our knowledge concerning this system, many points having been observed so often that they are beginning to be accepted as demonstrated facts.

My reason for presenting the following research may, if such reason be required, be briefly stated as follows :

It seemed to me desirable to confirm some of the observations previously made, with what seemed to me somewhat improved histological methods, hoping at the same time to throw new light on some of the still disputed questions, and especially to broaden our knowledge of these structures by systematically investigating the sympathetic ganglia of types from the several classes of vertebrates, using in each case the same methods.

During the investigation, material was obtained from the following vertebrates :

FISHES.

*Ambloplites rupestris* (Raf.), rock bass.  
*Micropterus dolomieu* (Raf.), small-mouth black bass.  
*Perca flavescens* (Mitch.), perch.

AMPHIBIA.

*Rana Catesbiana*.    *Rana* Hal.

REPTILIA.

*Chrysemys picta*.    *Chelydra serpentina*.  
*Emys melegaris*.

BIRDS.

*Gallus domesticus* — chicken.

MAMMALIA.

Dog, cat, rabbit, and guinea pig.

The subject-matter to be discussed in this research will be taken up as follows :

- 1) A brief discussion of the methods used.
- 2) A descriptive account of the results obtained in the preparations made from each of the above classes of vertebrates, in the order above named, with the literature bearing on the subject.
- 3) The general conclusions which may be drawn.

*Method.*

In the earlier portion of this work both the chrome silver and the methylene blue method were used ; the former was, however, soon discarded, owing to its greater uncertainty ;

largely, however, because in my hands more definite results were obtained with the methylene blue method. For the past two years the latter method alone has been used, and the results here presented are based exclusively on observations made with it.

Ehrlich's methylene blue method has so often been modified in one way or another, that, in order to discuss it at all, it seems quite imperative to take it up historically. Riese (1) has, however, collected all the literature bearing on this method, appearing before 1891; for the earlier modifications, therefore, the reader is referred to this summary. Since that time the most important modification of the method has been suggested by Bethe (2), who, in recommending the use of ammonium molybdate to convert the unstable methylene blue compound, as formed in fresh tissues, into one practically insoluble in water, alcohol, and the imbedding media, has given us a method by which methylene blue stained tissues may be imbedded in paraffin, sectioned and counterstained at will. Too much cannot be said for this important addition to the method suggested by Ehrlich.

The fixative suggested by Bethe has the following composition :

Ammonium molybdate . . . . .	1 grm.
Aqua dest. . . . .	10 c.cm.
Hydrogen peroxide . . . . .	1 c.cm.
Hydrochloric acid . . . . .	1 gtt.

Into this solution, cooled to + 2° C., the stained tissues are placed for four to five hours; are then washed in water, dehydrated in alcohol, and imbedded in paraffin. Meyer (4) suggests the omission of the hydrogen peroxide from the above formula, because, as he believes, it has a bleaching action — “etwas bleichend gewirkt hat.” Bethe (3) in a more recent communication has, however, drawn attention to the fact that this strong oxidizer is not, as such, present in the fixing fluid, because it at once unites with the *molybdänsauren ammonium*, and this has in the main lost the properties of the hydrogen peroxide. For a further discussion of this question the reader is referred to Bethe's (3) recent article. I may, however, add that before Meyer's paper came into my hands similar conclusions had



been reached by me; namely, that better results were obtained when the hydrogen peroxide was omitted from Bethe's formula. It may be stated that it has always been my plan to expose the ganglia, or other tissues to be stained, to the air, either in the animal or on the slide, until all the elements which it was thought would stain were clearly seen under the microscope; or, in other words, until the leukobase, the tetramethyldiamidothiodiphenylamin, which is formed by the living tissues when methylene blue comes in contact with them, is again oxidized, as it is in the presence of air. Hydrogen peroxide, or, as used in the formula, *hypermolybdänsauren ammonium*, would in such cases be unnecessary, and does, to some extent at least, as Meyer suggests, have a bleaching effect. I wish further to call attention to other modifications which Bethe (3) himself has suggested. He has tried to obviate the necessity of placing the tissues into an "ice-cool mixture," as his earlier method requires, by changing the unstable methylene blue stain, as found in fresh tissues, into one insoluble in water. To this end he places the fresh tissue in a saturated aqueous solution of ammonium picrate (suggested by Smirnow and Dogiel), and, at the expiration of fifteen to twenty minutes, transfers them to a solution prepared after one of several formulas, one of which is the following:

Ammonium molybdate . . . . .	1 gm.
Aqua dest. . . . .	20 c.cm.
Hydrochloric acid . . . . .	1 gtt.

Tissues two to three millimeters in thickness remain in this second solution three-fourths to one hour.

My experience with this modification of Bethe's earlier method has not been extended; as far as it goes, I have been led to conclude that the prefixation in ammonium picrate is open to a number of objections.

In the first place, picrate of ammonium seems, even in the short time the tissues are exposed to it, to act more or less as a macerating fluid, which is obviously an objection where the relation of end fibrillae to cells is to be investigated. Secondly, tissues prefixed in the picrate solution do not stain so readily as when the molybdate alone is used.

The methylene blue method, as used by me in this research, may be briefly described as follows: A 1%, 2%, or 4% solution of methylene blue (Grübler's rectificiert nach Ehrlich), made in a normal salt solution, was injected into a vein, easily accessible. In frogs, the large lateral cutaneous vein; in Reptilia, the jugular; in birds, the humeral (Owen); in Mammalia, the jugular or femoral, usually the former, were used. As to the percentage of the solution used, no definite statement can be made; the above solutions were used with equally good results in some experiments, and equally unsatisfactory results in others. In a general way it may be said that the stronger solutions stained more readily the cell body and branches of the sympathetic neurons, while the weaker solutions brought to view more clearly the pericellular plexuses; these statements are, however, open to many exceptions.

The quantity injected varied with the size of the animal used; from 2 to 4 c.cm. for a frog, to 60 to 80 c.cm. for a dog. The solution was allowed to flow into the circulation until the animal became blue, or until the heart's action was stopped.

Forty-five minutes to one hour after the injection, the ganglia or tissues to be examined were exposed; the larger ganglia, with the afferent and efferent nerves, were freed from the surrounding tissues, but not removed until they assumed a blue color, when they were excised and placed on a slide, and, if the staining seemed satisfactory, were placed in the fixative. Smaller ganglia were at once removed to a slide moistened with normal salt, and examined from time to time, until the stain was developed, when they were also placed in the fixative. The fixative used by me had the following composition:

Ammonium molybdate . . . . .	1 grm.
Aqua dest. . . . .	10 c.cm.
Hydrochloric acid . . . . .	1 gtt.

The molybdate is ground in a mortar and the water added; the solution is then removed to a flask and heated until perfectly clear; the hydrochloric acid is then added. The solution so made is placed in small glass jars, and these are surrounded

with snow or ice. The solution is best made some time before the injection, so that it may be properly cooled before using. In the fixative the tissues remain three to five hours. They are then washed in distilled water for about one hour and dehydrated and hardened in absolute alcohol, placed in xylol and imbedded in paraffin. As a rule, the ganglia were cut into serial sections, fixed to slide with the albumen fixative, and mounted in Canada balsam; other series were double-stained in alum carmine before mounting.

My experience as to the durability of preparations made in this way has not been the most satisfactory, although, as a rule, they have kept without fading for several months. Some of the sections (ganglia of Amphibia) have been well preserved for over two years, and are only now beginning to show signs of fading; others (sections of sympathetic ganglia of the chicken) began to fade at the end of several weeks. My experience would go to show that sections double-stained in alum carmine keep better than such as are stained only in the methylene blue. Why some sections should seem to fade more readily than others I am unable to say; it would seem, however, that sections thoroughly dehydrated and thoroughly washed in oil of bergamot and xylol fade less quickly than those where these precautions are not taken. Sections exposed to light fade more readily than those kept in the dark. Bethe (2) in discussing this phase of the matter says: "Die Haltbarkeit der Präparate, die mit der einfachen Fixation hergestellt sind, ist keine unbedingte. Sehr dicke Präparate zeigen oft schon nach zwei bis drei Monaten zuerst ein Dunkelwerden des Canadabalsams, auf den Trübung des Protoplasmas und Diffuswerden der Färbung folgt." "Schnitte halten sich besser, vielleicht deswegen, weil hier der Ueberschuss von Ammoniummolybdät, von dem in Verbindung mit Resten von Alkohol die Zerstörung auszugehen scheint, besser ausgewaschen wird."

In attempting to stain the sympathetic ganglia of fishes by injecting methylene blue into the circulation (through the caudal vein), it was found that the loose adipose tissue in the gill region, in front of the vertebral column, by the removal of which a number of small ganglia were brought to view, stained

so deeply that after an injection a search for them was usually fruitless. In fishes, therefore, it was found advisable to remove the ganglia, unstained, to a slide or watch crystal, and, following Dogiel's suggestion, to stain them in a  $\frac{1}{20}\%$  solution of methylene blue in normal salt. A portion of a ganglion thus treated was usually stained in forty-five minutes to one hour, the staining being controlled under the microscope. The tissues so stained were then fixed in ammonium molybdate, the further treatment being as above described.

In attempting to stain the tissues on the slide, I have tried a number of methods, which, in the hands of other investigators, have given good results. I may especially mention the methods recommended by Lawdowsky (5). He suggests the dilution of the methylene blue with one of the following solutions: Blood serum, egg albumen, a  $\frac{1}{10}-\frac{1}{5}-\frac{1}{2}\%$  solution of ammonium chlorate, or ferrum ammonium chloratum. The successful staining obtained by Lawdowsky has not been realized by me; on the contrary, results obtained with methylene blue diluted in normal salt have been much more satisfactory.

In closing the discussion of the methylene blue method, I need hardly add that, even with the greatest care, negative results are only too frequent. Why, when this method is used over and over again in exactly the same manner, in some instances successful staining is obtained, in others only failure, I am unable to say; but such is the case. Then, of course, it must be remembered that the investigator is always at a loss to know whether the preparation before him tells the whole story, or only a portion of it; whether, in other words, the structure before him is completely or only partially brought to view by the methylene blue.

#### SYMPATHETIC GANGLIA OF FISHES.

The sympathetic system of teleosts, to which subclass my investigations were confined, consists of a cephalic, a trunkal, and a caudal or post-anal portion, made up of a series of ganglia, united by intervening nerves into two cords lying close to the vertebral column. The cephalic portion extends forward



under the place of exit of the vagus, glosso-pharyngeal, and facial nerves, and sends branches to the trigeminus; at the point of union of the sympathetic with the cranial nerves mentioned, small sympathetic ganglia are usually found. At the anterior end of the trunk the two chains approach each other and present a relatively large ganglion — the splanchnic ganglion. In the trunk the two chains are found immediately under the vertebral column above the kidney, in which they may in part be imbedded. The caudal portion is found within the haemal arch, accompanying the aorta. The trunkal and caudal portions receive rami communicantes from the anterior roots of the spinal nerves. At the point of junction of the rami with the sympathetic cords small ganglia are found, which may be of microscopic size, or large enough to be recognized with the naked eye. This brief description of the sympathetic system of fishes is taken from Stanius's *Handbuch der Zootomie*, Zweiter Theil, Die Wirbelthiere, Zweite Auflage, pp. 143–146. The ganglia especially studied by me were the splanchnic and several small ganglia found in connection with the cardiac and intestinal branches of the vagus and the cells of Auerbach's plexus.

*Literature.* — The literature bearing on this portion of the subject is very meager. In the literature at my disposal I have found no observations on the structure of the larger sympathetic ganglia of fishes, made with the Golgi or methylene blue method. Monti (6) has used the Golgi method in studying the intestinal canal of teleosts with reference to its nerve supply, and has described small ganglia, composed of multipolar cells, possessing a single, unbranched axis-cylinder process. These ganglia take part in the formation of a plexus found in the submucosa, and from it fibers are given off which form a plexus in the muscularis mucosa. This latter plexus is continuous with a periglandular plexus, in which nerve cells, also with a single axis-cylinder process and surrounded with a very complicated network of fine fibrillae, are found. Sakusseff (7), a pupil of Dogiel, has stained the nerve plexus in the intestine of fishes with methylene blue. I was, however, not able to obtain the original article. Dogiel, in his brief reference to



this work, draws attention only to the fine fibers which leave the plexuses, passing through the mucosa to reach the epithelium.

The ganglia studied by me varied in size from such as were just recognizable with the naked eye to others about  $\frac{1}{2}$  mm. in their longest diameter. They were most often recognized as spindle-shaped swellings in the course of one of the vagus branches. They were removed to a slide and stained in  $\frac{1}{20}\%$  methylene blue solution in normal salt; fixed in ammonium molybdate and teased or sectioned, some of the sections being further stained in alum carmine. The ganglia are surrounded by a fibrous capsule, which is continuous with the perineural sheath of the nerves connected with the ganglion.

In sections, sympathetic nerve cells, medullated fibers, very small medullated fibers, and Remak's fibers may be seen.

*Shape and Structure of the Ganglion Cells.*—The shape of the nerve cells in the sympathetic ganglia of fishes varies. The cell body may be more or less regularly round or oval, and from it one or several processes may have their origin. The cells may therefore be unipolar or multipolar. In cells not too deeply stained in methylene blue the protoplasm appears granular, the granules staining more deeply than the remaining portion of the cell. The granules are very small, and evenly distributed through the protoplasm. The granules are probably the chromophile granules described for other nerve cells. In case the cell is deeply stained, it assumes a diffuse blue color, the granules showing only very indistinctly. In my preparations the nucleus was sometimes stained more deeply, again less deeply than the protoplasm, usually showing no distinct structure, although in preparations double-stained with alum carmine a nucleolus may now and then be made out. The cell body is invested in a nucleated capsule.

The neuraxis arises from a cone-shaped extension of the cell body. (See Pl. III, Fig. 1, *a-c*.) In sections it is sometimes difficult to make out with any degree of certainty which of the several processes is the neuraxis; for instance, *c*, of the above figure. In the neighborhood of the cell the neuraxis is non-medullated; whether at some distance from the cell it

becomes surrounded with a sheath of myelin, I have been unable to determine.

The dendrites vary in number; four to six are usually seen. They are quite short, and in my preparations do not undergo much branching; this of course may be due to imperfect staining. The dendrites terminate between the ganglion cells, are extra-capsular, and form a loose network, the arrangement of which depends in part on the number and relative position of the adjacent neurons.

*Medullated Fibers of the Ganglion.*—In sections a large number of medullated fibers are seen in the sympathetic ganglia of fishes. Many of these pass through the ganglia without giving off any branches. This is especially the case in the smaller ganglia, which are recognized as spindle-shaped enlargements in the course of a nerve trunk. Other medullated fibers, which give off one or several branches in the respective ganglion, are frequently met with. These branches, which are usually smaller than the parent fiber, end in a network on the cell bodies of the sympathetic cells. This network varies much in complexity; and in double-stained preparations it may easily be seen that this network is *intra-capsular*. It may be quite simple, as may be seen in Pl. III, Figs. 2 and 3, where only the network is shown. In Fig. 2, *a*, the portion of the neuraxis shown (a collateral branch of a medullated fiber), divides into four smaller branches, which anastomose to form a pericellular plexus; in Pl. III, Fig. 3, both the neuraxis and the terminal fibrillae are very small and beset with varicose enlargements. This pericellular plexus enclosed a long oval cell, lying between the nerve fibers of a ganglion; the cell was only faintly perceptible and is outlined in black; the processes were not made out. In Fig. 4 is shown a more complicated ending. The large axis cylinder, *a*, which was surrounded by a medullary sheath (not shown in the figure), breaks up within the capsule into a number of branches, some of which pass over the cell, others ending between the cell and the capsule in a number of branching, twisted, or coiled fibrillae. In Pl. III, Fig. 5, the same cell is again represented, after staining in alum carmine (the preparation from which Fig. 4 of Pl. III was sketched was broken

down, stained, and remounted). The latter figure may serve to show that the end fibrillae shown in Pl. III, Fig. 4, terminate between nuclei found within the capsule. In all instances (see also Pl. III, Figs. 6 and 7) where this more complicated ending was observed, the cell body of the sympathetic cell seemed imbedded in these nuclei; some of which, no doubt, belong to the cells of the capsule; others, if my observations are to be relied on, are within the capsule and seem to be grouped more particularly about that portion of the sympathetic cell from which the neuraxis arises. The number of the nuclei varies; in Pl. III, Fig. 6, sketched from a cell obtained by teasing a small ganglion, and which was completely isolated from the surrounding structures, I estimated that ten to twelve nuclei are within the capsule. The capsule enclosing this cell (*c*) was very clearly made out. The branches of the nerve fiber ending within the capsule are clearly shown in the figure, they alone being stained in méthylene blue, the other structures taking the alum carmine. In Pl. III, Fig. 5, above referred to, and in Pl. III, Fig. 7, the number of these nuclei is far greater. In the latter figure, which was also sketched from a teased preparation, may be seen the neuraxis of a large medullated fiber, from which two non-medullated collateral branches (*a'* and *a''*) are given off, these terminating within the capsule of the sympathetic ganglion cells (*A* and *B*) in a system of varicose end branches, the majority of which end between the intracapsular nuclei.

It may be of interest to note that Arndt (8) has diagrammed a cell which resembles very closely the one shown in Pl. III, Fig. 6. The cell referred to is reproduced by him in Pl. XIV, Fig. 38. Arndt, in his account, describes it as coming from a sympathetic ganglion of *Perca*, macerated in a  $\frac{1}{10}\%$  solution of acetic acid.

I have not been able to formulate any definite conclusions as to the nature of the nuclei above mentioned. I beg, however, to be allowed to give expression to a hypothesis which has often suggested itself in studying the preparations made from the sympathetic ganglia of fishes, but more particularly from similar preparations made from Reptilia; namely, that these

nuclei may belong to very much branched neuroglia cells, only the nuclei staining in the preparations at my disposal. This hypothesis is here only mentioned, and will be further discussed subsequently.

The medullated fibers ending in the pericellular plexuses may now and then be traced into some nerve root coming to the ganglion; their further course has not been ascertained.

A few very small medullated fibers are occasionally stained in sections of the sympathetic ganglia of fishes. My observations on these do not allow of any definite statement concerning them.

The non-medullated fibers seen in the ganglia are no doubt, to a large extent, the neuraxes of the sympathetic cells, constituting the ganglia, although in sections it is an exceedingly difficult task to trace such non-medullated fibers to a ganglion cell; I was able to do so only a few times. Small bundles of them may be traced into the nerves leaving the ganglia.

#### SYMPATHETIC GANGLIA OF AMPHIBIA.

The sympathetic system of the frog comprises a series of ganglia, lying on each side of the vertebral column, united by intervening nerves to form the ganglionated chains. The number of sympathetic ganglia usually corresponds to that of the spinal nerves, ten pairs of ganglia being found. Numerous smaller ganglia are found in the walls of the various organs — Bidder's and other ganglia in the heart; ganglia in the lungs, the pharynx, the intestinal canal, and the bladder. Sections were usually made of the larger ganglia — the first and second (this being the largest), and seventh, eighth, and ninth. The last three, on account of their size, the length of their rami, and their exposed position, are very easily found.

The methylene blue method was used to the exclusion of other methods. The stain was injected into the circulation of the living frog. The larger ganglia were fixed in ammonium molybdate, sectioned, and some of the sections double-stained in alum carmine. The smaller ganglia were fixed in ammonium picrate and cleared in glycerine.

















*Literature.* — The ganglion cells of the frog's sympathetic were first described by Beale and Arnold. In Beale's (9) account the following statement may be found: "In the fully formed cell a fiber comes from the center of the cell (straight fiber), and one or more fibers (spiral fibers) proceed from its surface." "These are wound spirally around the first fiber." Arnold (10) described similar bodies in connection with the nerves in the frog's lung, believing them to be peculiar to this organ. In Arnold's (11, 12) later publications he states that these cells were found generally in the sympathetic system of the frog, and gives a much more detailed account of the straight and the spiral processes. The straight fiber he was able to trace through the protoplasm of the cell to the nucleolus, while the spiral fiber had its origin in a network of fine fibrillae connected with the branches of the nucleolus. (See Pl. I, Figs. 3, 4, and 6, Virchow's *Archive*, Vol. XXXII.) Courvoisier (13) corroborated many of the observations made by Arnold. His results may be summed up as follows: The straight fiber begins or ends in the nucleus, while the spiral fiber has its origin in a network which is connected to the nucleolus by "root fibers" — *Wurzelfäden*. This network gives origin also to "commissural fibers," which connect the network of adjacent cells. This network was also described by Kollman and Arnstein (14). On the other hand, Schwalbe (15) stated that the sympathetic ganglia of frogs contained two kinds of cells — bipolar cells, with straight and spiral fibers, the latter arising from the cell body and making only a few turns around the straight fiber, and unipolar cells, with only the straight process, with a prominent capsule, the thickenings or folds of which may simulate a spiral fiber. This latter form Schwalbe believed to be the more common.

Arndt (16), while not denying the nervous nature of the spiral fiber, suggests that the fibrillar structure of the capsule may in some instances be mistaken for a spiral fiber, inasmuch as the fibrillae of the capsule are now and then arranged transversely to the axis of the straight fiber as it leaves the cell.

Axel, Key, and Retzius (17) made further contributions by showing that the spiral fiber became invested with a medullary

sheath at some distance from the cell body; thus for the first time pointing out conclusively its nervous nature.

The true nature of the spiral fiber and the network, which had been described by some of the earlier investigators, and their relation to the protoplasm and the straight process of the cell, were, however, not understood until Ehrlich's methylene blue method became known. Ehrlich (18), in his first communication on the reaction of methylene blue on living nerve tissues, gives a very clear description of the spiral fiber and network. I take the following brief account from this article: "The spiral fiber, which alone was stained blue, divides into fine fibrillae, which unite to form a network, which network may enclose the entire cell or only a portion of the cell. From this network fine fibrillae are given off, which end on the cell in small terminal swellings. This mode of ending of the spiral fiber was observed in all the larger ganglia examined; also in the smaller ganglia found in the heart, bladder, lung, and pharynx." The fact that the spiral fiber was medullated led Ehrlich to believe that it was a cerebro-spinal fiber, and that its course was centralward, and to express the opinion that it conveyed impulses from the nerve centers to the sympathetic ganglia through the network about the cell. Ehrlich's observations were soon corroborated by Aronson (19), and by Arnstein and his pupil Smirnow. In the account given by Arnstein (20) the network formed by the end branches of the spiral fiber is described as a closed one; the terminal fibrillae mentioned by Ehrlich and Aronson were only occasionally seen, and then only when the network was imperfectly stained. Arnstein states that they found it extremely difficult to determine which direction—centralward or toward the periphery—the spiral fiber takes on leaving the cell; often it was impossible to decide. In the sympathetic cells found in the pharynx of the frog, the spiral fiber went to the periphery, often branching in its course. Retzius (21), in a short but very comprehensive account of his observations on methylene blue stained sympathetic ganglia of the frog, adds a number of details to observations previously made. He here points out that the terminal network of the spiral fiber is always within the capsule of the

ganglion cell. The network is described as a closed one with varicose fibrillae. At the nodal points of the network, nodular enlargements are often seen; these vary in shape and number. He further observed that the spiral fiber became myelated at some distance from the cell, and that it often divided into two branches, — "*tubes en T*," — the two arms being often traced in divergent directions. (See Fig. 3 of his article.) Retzius looks upon the spiral fiber as of cerebro-spinal origin, and reaches the following conclusions as to its function: "Nachdem die Spiralfasern sich getheilt haben, schicken sie den einen Arm an eine sympathische Ganglienzelle, um in dieser Weise eine Verbindung mit ihr einzugehen."

Lawdowsky (22) (whose account I have not been able to obtain) and Feist (23) have also written on the frog's sympathetic. The latter's observations corroborate in the main those made before him; he finds, however, a different explanation for the network and spiral fiber. Feist, while admitting that the spiral and network often stain alike, does not regard this as sufficient reason for regarding them as identical in structure. The conditions, he says, may just as well be the following: "The ganglion cell is united to the capsule through a layer of protoplasm, arranged in the form of a network. The spiral fiber, as it approaches the cell, pierces the protoplasmic network at some nodal point and then enters the substance of the cell."

Feist (23) also finds, as his Figs. 20, 21, and especially 22, may show, that the straight process undergoes division at some distance from the cell. As a result of his investigations he reaches the following conclusion, which is so at variance with results previously obtained that it will be given in full: "Nach dieser Beobachtung rückt die bipolare Ganglienzelle des Froschsympathicus in das Schema der multipolaren Hirn- und Rückenmarksganglienzellen der Säuger ein, nur mit dem Unterschiede, dass bei jener die verästelten Fortsätze nahe bei einander von einer weit vom Centrum der Zelle entfernten Stelle, der Theilungsstelle der geraden Faser, abgehen, welche letztere hiernach nur eine lang ausgezogene Partie der Zellsubstanz darstellt." If I understand Feist correctly, the spiral

process would thus become the neuraxis of the cell, the straight process, with its branches, the dendrites; a conviction entirely at variance with the facts in the case, as will be subsequently shown.

Before concluding the review of the literature bearing on the structure of the sympathetic ganglia of Amphibia, mention must be made of Smirnow's (24) later publications on the subject. I wish, however, at this place to draw attention only to that portion of his writings in which he discusses the relation of the network to the spiral fiber and its further course after leaving the cell.

According to Smirnow, the spiral fibers extend toward the periphery, and are, according to the location of the nerve cell, distributed to various peripheral tissues. "They form anastomoses with other cells, in so far as they may join the processes of other cells; or may become continuous with the network enclosing other cells; or they may be distributed to heart muscle or form vasomotor fibers to the blood vessels. The distribution of the spiral fibers is such that, as a rule, a portion of it serves to form anastomoses with other cells, while the remaining portion goes to muscle tissue or to blood vessels."

Nearly all writers who, after Ehrlich, have used the methylene blue method in their investigation of the sympathetic ganglia of Amphibia, are agreed that the spiral fibers and the network enclosing the cell body are continuous, and that they stain more readily than do the other structures of the ganglion. There exists, however, a difference of opinion as to the nature of the spiral fibers; Ehrlich (18), Aronson (19), and Retzius (21) looking upon them as cerebro-spinal fibers, while Arnstein (20), Smirnow (24), and Feist (23) ascribe to them other properties.

The results obtained by the writer will be taken up as follows:

- 1) Sympathetic ganglion cell of Amphibia, its structure, the straight process and the capsule of the cell.
- 2) The spiral fibers and the pericellular network and the course of the spiral fiber after it leaves the straight process.

*Sympathetic Ganglion Cell of Amphibia.* — It may be stated at the outstart that the writer regards the sympathetic neuron



of Amphibia as a unipolar cell, adopting in this respect Kölliker's (25) view. In methylene blue stained preparations the cell body of such neurons remains, as a rule, wholly unstained. In such sections, double-stained in alum carmine, its outline may, however, be clearly made out, as Pl. III, Figs. 9 and 10, may show.

In such sections it may be seen that the cell body is more or less regularly round or oval, becoming attenuated at one end, from which portion arises the straight process, *a*, of Pl. III, Figs. 9 and 10 respectively. The straight process often remains unstained; in some cells, however, it is stained faintly blue, and in such cases it gradually loses its blue color as it approaches the cell body, becoming continuous with the drawn-out portion of the cell. Smirnow (24) states that the straight process may in some cases take on a medullary sheath (see Fig. 16 of Smirnow's article). This I have not seen. In sections only relatively short, straight processes are seen; they are not, as far as my observations go, medullated near the cell body. In sections of the nerve roots coming from the sympathetic ganglia, very small medullated fibers may now and then be seen, no doubt medullated sympathetic fibers such as Smirnow describes, and as were seen many years ago by Kölliker and others in the sympathetic nerves of the frog. The great majority of the sympathetic fibers of the frog are, however, non-medullated. Schwalbe, Feist, and Smirnow state that the straight process may undergo branching at a variable distance from the cell body. The former (Schwalbe and Feist) regard the straight process as a dendrite, if I understand Feist correctly, as has been stated in the review of the literature. Smirnow (24) likens the branching seen by him (Fig. 11 of his article) to a "T"-shaped process, as seen in the spinal ganglia. He leaves it an open question whether all straight processes divide in this way.

In sections of larger ganglia such a division was never seen by me. The straight process is here, as a rule, so indistinctly stained that it could not be traced for any distance. In surface preparations of the bladder and pharynx, stained, *intra vitam*, in methylene blue, fixed in ammonium picrate, and cleared in



glycerine, I was, however, able in a number of instances to trace the straight process of a sympathetic cell for some distance from the cell body, and in one preparation of the pharyngeal mucous membrane and the oesophagus of a frog prepared as above stated, a straight process coming from a sympathetic cell, which divided into two branches, was clearly made out. These branches could be traced toward a small gland situated in the upper part of the oesophagus, where both underwent a second division; from here one of the resulting branches could be traced on to one of the alveoli of the gland, where it was lost to view. The appearance here presented seemed to indicate that the straight process extended toward the periphery, and in this case innervated the small gland above mentioned, although the ultimate endings on the gland cells could not be made out. In the few cases seen by me, where the straight process branched, this did not simulate a "T"-shaped branching as described by Smirnow; the branching was usually at an acute angle. The arrangement seen by him I should regard as accidental. The observations made by me led to the conclusion that the straight process is the neuraxis of the unipolar sympathetic cell of Amphibia, innervating gland and non-striped muscle tissue, as my observations on the bladder of the frog would show. This conclusion is, therefore, wholly in accord with the following statement made by Kölliker (25): "Die geraden Fasern sind nicht Dendriten oder Protoplasmafortsätzen zu vergleichen, sondern einfach Achsencylinderfortsätze der betreffenden Zellen, die zu Muskelfasern treten und hierbei Verästelungen zeigen."

The statement has been made that the sympathetic neurons of Amphibia were unipolar cells. As an exception to this, I may say that the sympathetic cells found in the wall of the intestinal canal (stomach, small and large intestine) of frogs are distinctly multipolar, as the two cells reproduced in Pl. III, Fig. 11, may show. These cells were sketched from a preparation of the large intestine of a frog, stained, *intra vitam*, in methylene blue, and fixed in ammonium picrate; in the figure the purplish color assumed by methylene blue stained tissue when fixed in the picrate is not reproduced. The cell body of

these neurons is very irregular; the neuraxis, *a*, which in the cells sketched could be traced for a long distance as an exceedingly fine fiber, was lost in the network of Auerbach's plexus. The dendrites, *b*, have a very irregular, jagged appearance, perhaps better shown in the figure than can be described. Such multipolar cells were seen only in the intestinal canal; in all other sympathetic ganglia of the frog known to me the cells are unipolar.

*Pericellular Network and Spiral Fiber.*—In well-stained methylene blue preparations of any sympathetic ganglion of a frog it may be seen that the cell body of each neuron constituting said ganglion is enclosed in a pericellular network or plexus. In such preparations, double-stained in alum carmine, it may readily be seen that this network is intra-capsular (see Pl. III, Figs. 9 and 10, *c*, capsule of cells), and is in contiguity with the enclosed cell body, but never continuous with it. The arrangement and structure of the fibrillae constituting the network are very variable, as may be seen from Pl. III, Figs. 8–10. The fibrillae may be very loosely or more densely woven; their arrangement seems largely accidental. The fibrillae may be smooth or varicose. This apparent difference in structure seems to depend somewhat on the length of time the tissues are exposed to the air before fixing them. It will be remembered that with this method the nerve tissues are usually almost colorless about one hour after the injection, the interval of time allowed to elapse before the ganglia are exposed; if, on exposing the ganglion to the air, they become blue in a few moments, as they often do, the fibrillae of the network have a much smoother appearance than when it is necessary to expose them ten, fifteen, or even twenty minutes before the stain has developed, in which case the fibrillae usually present a very varicose appearance. Now and then quite large nodular masses, stained deeply blue, may be seen at some nodal points in the pericellular network, *d*, of Pl. III, Figs. 8 and 9; Retzius (21) has also described such nodular enlargements. The question as to whether the network is an open one, as described by Ehrlich (18), Aronson (19), and Lawdowsky (22), or a closed one, — Arnstein (20), Smirnow (24), and Feist (23), — has, I believe, been most cor-

rectly answered by Retzius (21), who states that, as a rule, the fibrillae form a closed network, but that now and then free-ending fibrillae may be observed. In Pl. III, Fig. 8, which represents a portion of a section of the sympathetic ganglion on the second spinal nerve of a frog, in which the structures were very well stained in methylene blue, I have indicated by the letter *e* a number of instances where fibrillae seem to end free, usually in a small nodular enlargement. That the network is continuous with the spiral fiber seems evident to all investigators, with the exception of Feist (23), who have studied the sympathetic ganglia of Amphibia with the methylene blue stain. Most often the network and spiral are alone stained, and in sections of 20  $\mu$  to 40  $\mu$  thickness the spiral fiber may often be traced with the utmost clearness through the "granular mass" into the fibrillae of the network; the granular mass, *g*, of Pl. III, Figs. 8-10, forming a more densely woven portion of the network, as has been pointed out by Retzius (21).

My own observations on the course of the spiral fiber after it leaves the straight process lead me to conclude that its course is centralward; or, to express it in another form, the pericellular plexus and spiral fiber are parts of a neuron, the cell body of which is situated more centrally than the respective sympathetic ganglia, in which the structures are found. These conclusions are based on observations made on ganglia prepared in the following way: a small quantity ( $\frac{1}{2}$  to 1 c.cm.) of a 1% solution of methylene blue was injected into the circulation of a small frog. At the expiration of about an hour the entire sympathetic system was exposed. The entire sympathetic chain on one side, with the rami communicantes and segments of the spinal nerves from which they had their origin, was then removed and placed on a slide moistened with normal salt. As soon as the stain seemed developed as much as it would, the preparation was fixed in ammonium picrate and cleared in glycerine. When properly fixed and cleared, the entire chain was placed on a slide, where all the surrounding tissues were removed with teasing needles, care being taken not to tear any of the rami from the ganglia. The entire chain, thus freed from all connective tissue, was then mounted in ammonium

picrate and glycerine. By injecting only a small quantity of the methylene blue stain, as above stated, now and then only a few nerve fibers were stained in some of the ganglia of the chain, the ganglia clearing in the glycerine to such an extent that such fibers could be followed for long distances, even under the oil immersion. In such preparations it was often possible to trace a medullated fiber from a white ramus into a ganglion, or from the interganglionic nerve trunk into the respective sympathetic ganglion, and through several branchings to its ending in a spiral and pericellular network.

Some of my best examples are reproduced in Fig. I. I may say that the fibers here sketched were drawn under a  $\frac{1}{12}$  in. oil immersion lens, with the camera lucida, and drawings reduced 5 or  $2\frac{1}{2}$  times, as stated in the description of the figure. In the above figure,  $x$  by the side of a fiber indicates a node of Ranvier, and  $y$  the change of a medullated to a non-medullated fiber. In fiber *A* is shown the neuraxis of a large medullated fiber, which entered the first sympathetic ganglion through a nerve uniting the ganglion with the vagus ganglion; the fiber was traced for a distance much greater than shown in the figure. Soon after entering the ganglion this nerve divided into two branches, both of which were medullated. The right branch was traced through three nodes of Ranvier, when it became non-medullated. After a short distance this non-medullated fiber divided into two branches, one of which was traced into a spiral fiber and pericellular basket; the other terminated abruptly. The left branch of fiber *A* was traced through four nodes of Ranvier, where a small collateral branch was given off ( $b$  in the figure), which could be followed for only a short distance. At the succeeding node the fiber became non-medullated and divided into three branches,  $c$ ,  $d$ , and  $e$ . Branch  $c$  was traced to a pericellular network; branch  $d$ , reflected upon itself at  $d'$ , divided into two branches, one of which ended in a spiral and network; branch  $e$  was followed a long distance, when it also terminated in a spiral and pericellular network. Fibers *B*, *C*, and *D* are sketched from the eighth sympathetic ganglion. In each case the fiber sketched shows only a short segment of the neuraxis before branching; these



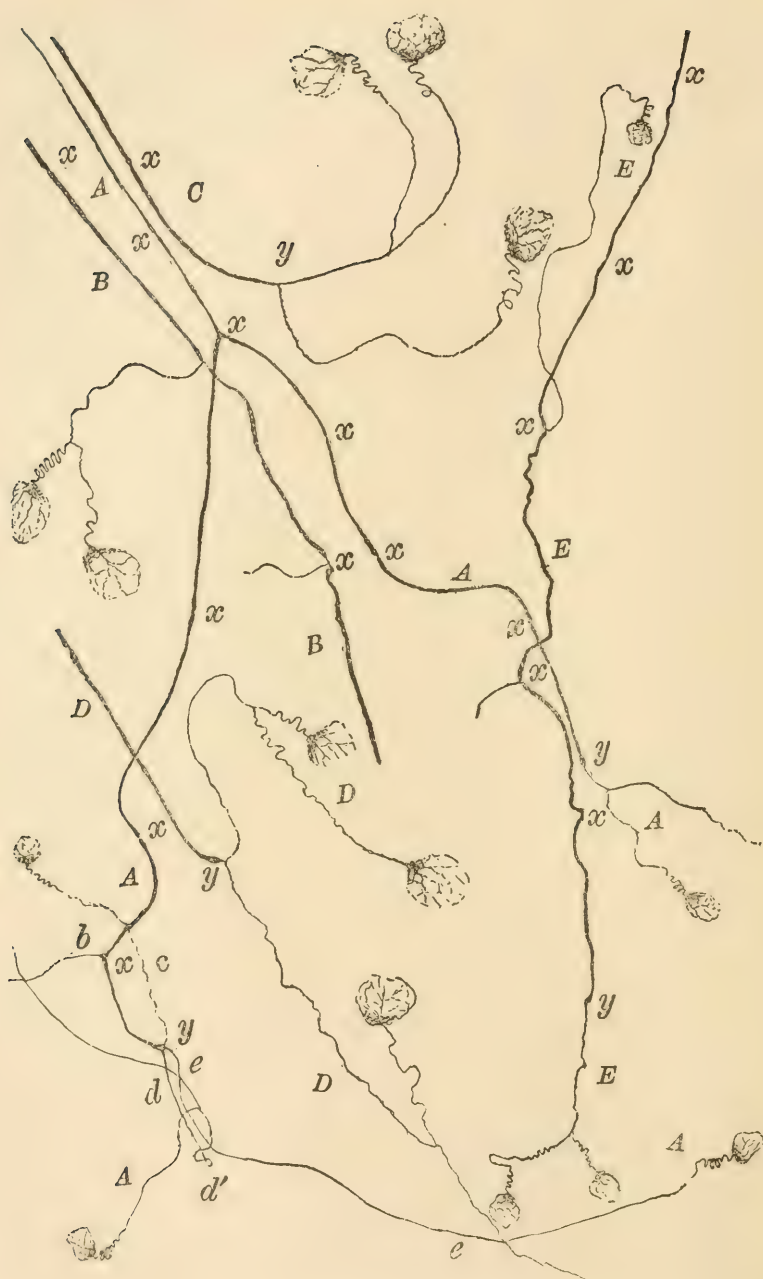


FIG. I. — Spiral fibers from sympathetic ganglia of frog. (See text for description.)



were traced in every instance into a white ramus of the ganglion.

Fiber *E* may be explained somewhat more fully, as it explains, I believe, some observations made by Retzius (21) and Smirnow (24). This fiber entered the eighth sympathetic ganglion through a white ramus, and could be traced through a number of nodes of Ranvier, only two of which are shown. At the second node shown in the figure there was given off a non-medullated collateral branch, which terminated in a spiral and pericellular plexus; two nodes further on the fiber became non-medullated, ultimately branching into two branches, each of which terminated in a spiral and plexus. In a teased preparation, showing only the upper portion of the fiber, it may easily be seen how one might be led to say that the spiral fiber branched "*en T*," as Retzius (21) has done, and diagrammed in Fig. 3 of his article. Arnstein (20) and Smirnow (24) have also described a branching of the spiral fibers; they, it will be remembered, assume that the spiral fiber has a peripheral course. Such observations were made, I believe, on only partially stained fibers. Whenever I was able to trace a spiral fiber to a medullated fiber which had a peripheral course, such medullated fiber always terminated in a spiral in some more distal portion of the ganglion.

The anastomosis between pericellular baskets of adjacent ganglion cells, described by Smirnow (24) and long ago mentioned by Courvoisier (13) and others, I have never seen in sections. In methylene blue preparations of whole ganglia, cleared in glycerine, I have now and then seen what might be regarded as an anastomosis between two baskets. It would not, it seems to me, deserve the importance given it by Smirnow (24).

The observations above mentioned seem to me to present very strong evidence in favor of the hypothesis long ago expressed by Ehrlich (18) and Retzius (21), namely, that the spiral fiber was the ending of a cerebro-spinal fiber. They seem to me to show also that the course of the spiral fiber, after it leaves the straight process, is centralward. I have attempted by the degeneration method to determine this more clearly by

experiments of the following nature: the spinal cord of frogs was destroyed by inserting a hot needle through the peripheral end of the spinal canal; anaesthetized frogs stand this very well. In the frogs so operated upon, the posterior extremities seemed entirely paralyzed. At the end of five, ten, and twenty-five days a certain number of frogs were injected with methylene blue, with a view of staining the sympathetic ganglia. It was found, however, that even in frogs in which a portion of the cord had been destroyed twenty-five days previous to the injection, the nerves were not as yet degenerated; even the motor endings, in the sartorius, for instance, could be stained very easily. The sacral sympathetic ganglia showed, in all but one instance, the spiral fibers. This experiment (of ten days' duration) seemed at first conclusive, as only a few spiral fibers and baskets were stained; the unipolar ganglion cells stained much more readily than usual. Later experiments, where a longer time elapsed between the primary operation and the injection, did not, however, corroborate the above result. It may be stated that not much reliance can be placed on these experiments, as they were made on "winter frogs" in the early spring, at a time when it is well known that their metabolic changes are at their lowest. This, I think, may account for the fact that no degeneration of nerves was observed, dissection showing that the posterior portion of the spinal cord was completely destroyed. It is my aim to repeat such experiments on more suitable frogs.

The following brief summary of the results obtained in the study of the sympathetic ganglia of Amphibia may here be in place:

- 1) The sympathetic neurons of the frog are unipolar cells, the neuraxes of which (straight processes) innervate involuntary muscle and gland tissue (Kölliker).

- 2) Entering the sympathetic ganglia through the white rami, or through the interganglionic nerves, are seen medullated fibers, probably of cerebro-spinal origin, which fibers divide in the ganglion into two, possibly three, fibers, also medullated; at various places in the course of these fibers non-medullated, collateral branches are given off, which, with or without further

branching, end in spiral fibers and pericellular plexuses. After a longer or shorter course in the ganglion, the medullated branches lose their medullary sheath, and, as non-medullated fibers, also end in spiral and pericellular plexuses.

3) The pericellular plexuses surround the cell bodies of the sympathetic neurons, and are always intra-capsular. They represent the ending of cerebro-spinal fibers in the ganglion.

#### SYMPATHETIC GANGLIA OF REPTILIA.

In the tortoise the sympathetic chain begins in the superior cervical ganglion. The nerve connecting this ganglion with the succeeding one runs in close relation to the pneumogastric to about the middle of the neck, where it leaves the nerve, and, passing outwards and backwards, reaches the middle cervical ganglion; in *Chelydra serpentina*, a ganglion nearly a centimeter in length. From this ganglion several branches are given off, the largest of which passes toward the axilla and ends in the inferior cervical ganglion. Crossing the brachial plexus, the nerve which forms the continuation of the cord ends in the stellate ganglion. The relative size of these three ganglia differs in the several species studied; also, to some extent, in the same species. They are all, however, relatively large, and for this reason were used almost exclusively. They may be readily exposed by removing the plastron and pushing aside the tissues of the neck. From the stellate ganglion two filaments run back, one on each side of the subclavian artery, to a small ganglion which lies on the first dorsal nerve. In the succeeding dorsal segments are found small sympathetic ganglia, resting on the spinal ganglia. The sympathetic cord then runs back and becomes connected with sacral nerves. The two chains may then be traced as a single nerve into the tail, a number of small ganglia being found here. The account of the sympathetic system of the tortoise has been taken, with almost no alteration, from Martin and Moal's *Handbook of Vertebrate Dissection*, Part I: How to Dissect a Chelonian. Their description has reference more especially to the red-bellied slider terrapin (*Pseudemys rugosa*); their description

has been followed, as a number of dissections of the species worked on by me seem to agree with it in all essentials.

The sympathetic ganglia in the pharynx, oesophagus, in the heart (see Gaskall, *Journal of Physiology*, Vol. V), in the wall of the stomach and small intestine, were also studied. The *intra vitam* methylene blue method was alone used. The ganglia were fixed in ammonium molybdate, sectioned, and some of the sections mounted at once in balsam; others were double-stained in alum carmine before mounting in balsam.

In the literature at my disposal I have not seen any reference to observations made on the sympathetic ganglia of Reptilia with either the methylene blue or the chrome silver method. The older literature was not searched carefully enough to say whether these structures have been examined at all; this did not seem necessary, as such references would have no direct bearing on the observations here recorded.

*Sympathetic Neurons of Reptilia: Tortoise.* — The sympathetic neurons of the Reptilia studied vary greatly in shape and size. Their shape may be multipolar, bipolar, or unipolar. Multipolar cells are found in all ganglia, and in well-stained cells the dendrites and neuraxes can usually be differentiated. Only one neuraxis is found; this may arise from the cell body, as is the usual way, or from a dendrite at some distance from the cell body.

Bipolar cells are not numerous, and are usually found near one of the poles of a ganglion, between the efferent or afferent nerve fibers. Such cells are now and then seen in peripheral nerves some distance from a ganglion; they are now and then seen in the nerves found in the oesophageal mucosa. Cells which I have classed as unipolar belong to the largest cells seen; their peculiarity calls for a somewhat more extended description. These cells belong to the largest cells found; they are much more numerous in the larger ganglia of the chain, although they have often been recognized in many of the smaller ganglia. The cell body of such cells (see Fig. II, and Pl. IV, Fig. 12) is usually more or less regularly round or oval, and very often presents a depression at one side of the cell body, from which arises one large process. This process















varies greatly in shape and in the course it assumes after leaving the cell body. It may be quite straight (Fig. II, *A*, and Pl. IV, Fig. 12, *A* and *B*), and break up at a variable distance from the cell into secondary branches; or it may assume a tortuous course (Fig. II, *B*, *C*, and *E*), and may (see figure) be partly wound around the cell or twisted upon itself. This

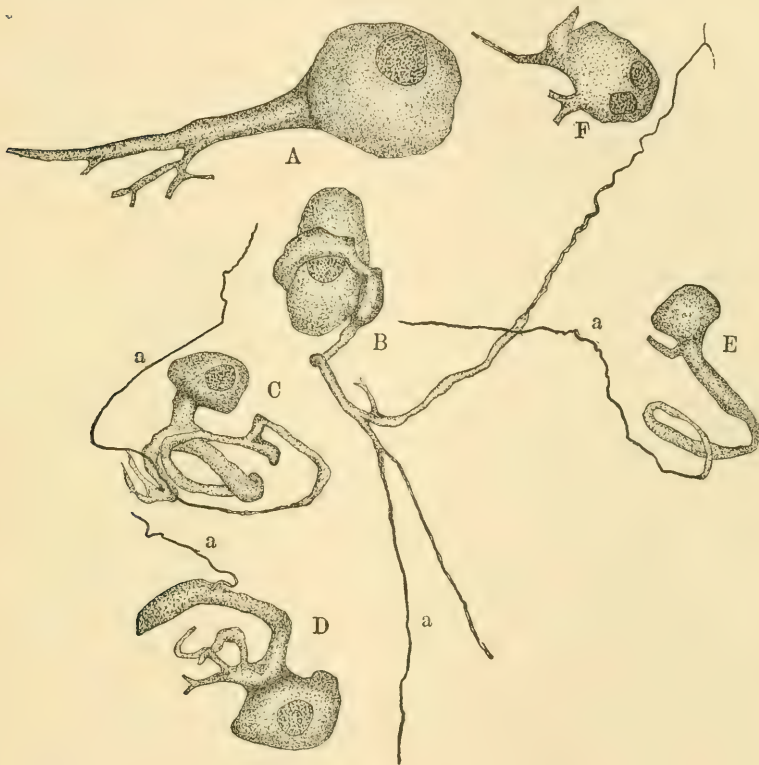


FIG. II. — Sympathetic neurons of Reptilia. (See text for description.)

process may again be quite short (Fig. II, *D*), and break up at once into a number of large secondary branches. In such cells the neuraxis may arise directly from this large process, *a*, of cell *B*, Pl. IV, Fig. 12, and *a*, of cells *B*, *C*, and *E*, of Fig. II, in which case the large process becomes gradually attenuated and stains more deeply; or it may arise from one of the dendritic branches at some distance from the cell body,



as in cell *A*, Fig. 12 (in which case the neuraxis was recognized by a nucleus of the sheath of Schwann, *b*, of figure), or again, cell *D* of Fig. II, where the neuraxis was recognized by its greater affinity to the methylene blue. It should be stated that the figures here reproduced were sketched from sections of sympathetic ganglia of *Chrysemys picta* and *Chelydra serpentina*, which accounts for the abrupt ending of some of the processes. The facts here given have been abundantly verified on fresh tissues.

In methylene blue stained preparations of sympathetic ganglia of Reptilia, examined fresh or in sections of such ganglia fixed in ammonium molybdate, the chromophile granules of the protoplasm are clearly seen. These granules vary in shape and size, and are distributed more or less evenly through the cell protoplasm, leaving free, however, a narrow zone immediately surrounding the nucleus, as may be seen in Pl. IV, Fig. 12.

The cell body of the sympathetic neuron of Reptilia is surrounded by a nucleated capsule, which seems continuous with the neurolemma of the neuraxis of said cell; although my observations on this point are not conclusive.

Within the capsule of many of the sympathetic cells (and this is especially true of the large unipolar cells above described) are found a varying number of nuclei, the nature of which has not been clearly determined. They are always recognized in sections of ganglia stained in methylene blue and double-stained in alum carmine; they may, however, also be seen in sections of ganglia hardened and stained in the more usual methods. In discussing similar nuclei found within the capsule surrounding the sympathetic ganglion cells of fishes, it was mentioned that these nuclei were looked upon as nuclei of neuroglia cells. Cajal has described cells which he regards as neuroglia cells in the superior cervical ganglion, and Dogiel (40) has drawn attention to spindle-shaped or star-shaped cells with long branching processes found in the sympathetic ganglia of Mammalia. Such cells, stained in methylene blue, are shown by Dogiel in Pl. XIV, Fig. 15. They are described as forming a network outside of the capsule of the ganglion cells. By reason of the fact that neuroglia cells are not stained in methylene blue,

Dogiel is inclined to think that the cells described by him do not belong to neuroglia tissue. He likens them to the branched connective tissue elements, which are found on the membrana propria of gland alveoli, which, as he states, are also occasionally stained in methylene blue. The cells in question have never been stained by me in methylene blue. This may be due to the fact that in all my work the methylene blue solution was injected into the living animal, in which case, as is well known, many elements do not stain as readily as when the methylene blue solution is added directly to the freshly removed tissues. My only reason for suggesting that the nuclei found within the capsule of many of the sympathetic cells of Reptilia are nuclei of neuroglia cells is based on some observations made on ganglia hardened in Müller's fluid, imbedded in paraffin and sectioned, the sections being then stained in haematoxylin and double-stained in the acid fuchsin—picric acid solution suggested by Van Gieson. In such sections are seen a large number of fine fibrillae, which seem to be more or less intimately connected with the nuclei above mentioned. These fibrillae are not, I believe, nerve fibrillae, belonging to the pericellular plexus presently to be described; their smooth and regular contour and their apparent connection with the nuclei would exclude such a supposition. Time has not been at my disposal to consider these nuclei more fully. I mention the above hypothesis in the hope that it may stimulate further inquiry into their structure.

In the sympathetic ganglia of Reptilia, stained in methylene blue, medullated nerve fibers, which enter the ganglia through their rami, are abundantly found. Many of these medullated fibers have been traced into intra-capsular, pericellular plexuses. Such pericellular plexuses may be described under two heads:

a) Simple pericellular plexuses, without spiral fibers, surrounding, as a rule, the cell body of multipolar or bipolar ganglion cells.

b) Complex pericellular plexuses, with spiral fibers, more often found surrounding the cell body of the large unipolar cells above mentioned.

The pericellular plexuses of the simpler type resemble very closely those to be described for birds and mammals. They are found in all sympathetic ganglia, but are relatively more numerous in the smaller ganglia. When the shape of the enclosed cell can be ascertained, it will, as a rule, be found to be multipolar. But as the sympathetic cells are generally only imperfectly stained, when the pericellular plexuses are well stained, the enclosed cells are usually not clearly made out. The above statement may, therefore, be open to exceptions. In Pl. IV, Fig. 13, three of the simpler pericellular plexuses are sketched. In each case the non-medullated axis-cylinder (a collateral branch or end branch of a medullated fiber, as one may occasionally trace such a branch to a medullated fiber) breaks up, just before or after it pierces the capsule (not shown in the figures), into several secondary branches, which, after further division, are woven into a network varying greatly in complexity and arrangement. The fibrillae of the network are often very varicose, showing quite large nodular enlargements. This network or plexus is generally seen as a closed one; only here and there some of the fibrillae seem to end free. (See *b* of Fig. 13.) These plexuses are always intra-capsular, and surround the cell body of a sympathetic cell; the capsule, though often not stained, may be recognized if much of the light is cut off.

The more complex pericellular plexuses vary greatly in structure; they are usually found, as above stated, surrounding the large unipolar cells. Their structure may best be made clear by reference to a number of figures showing some of the different types found. In Pl. IV, Fig. 16, the relation of such an end plexus to the enclosed ganglion cell and capsule is well shown. The axis-cylinder of the nerve fiber (*a*) ending in the plexus breaks up into several branches, which are wound around the single process of the sympathetic cell enclosed in its end plexus. From these spirally arranged branches secondary branches are given off, which, on approaching the cell body of the sympathetic cell, are woven into a loose basket-like network. Very often the axis-cylinder ending in such pericellular plexuses is spirally wound around the process of the sympa-

thetic cell before breaking up into secondary branches. The number of such spirals and their arrangement are subject to much variation. In Fig. 14 an axis-cylinder with four spiral turns is shown. In Fig. 15 is shown a long spiral, which surrounds a long and straight process of a unipolar sympathetic ganglion cell. In Pl. IV, Fig. 17, we may see a portion of four pericellular plexuses; special attention is, however, drawn to the one in connection with the axis-cylinder showing the spiral turns. The spiral fiber, *a*, here shown is a collateral branch of a medullated fiber, *a'*; the collateral branch, after making many turns around the process of the enclosed sympathetic cell (the process and cell body of the sympathetic cell are not shown in the figure; they can, however, be quite clearly made out in the section), breaks up into a number of fine varicose fibrillae, which form the pericellular plexus. Attention needs further to be drawn to the network of fine fibrillae seen within the spiral fiber. Many of these fine fibers branch from the spiral fiber. Their arrangement is, however, so complex that they can be followed for only a short distance. Such intra-spiral fibers were seen only a very few times, when the preparations seemed very well stained. They form a network which is in very close proximity to the straight process of the enclosed sympathetic cell, and suggest the possibility that the process may be stimulated directly through the intra-spiral fibrillae. This figure shows further some very small varicose fibers (*c*), which enter the spiral from without. Where these fibers come from, and the nature of their ending, I have not been able to ascertain. I should like to suggest that such fibers may be sympathetic fibers ending on the sympathetic cell or on one of its large processes. I possess, however, very few observations that would tend to strengthen such a view. My reason for suggesting the hypothesis is based on the fact that, in birds and Mammalia, end branches of sympathetic fibers would in some cases seem to end on dendritic branches of other sympathetic cells, as will be stated in the following pages. In Pl. IV, Figs. 14-16, only the spiral fiber and the pericellular plexuses are shown; their arrangement and structure are more easily seen when the other parts of the ganglion are



not sketched. In Pl. IV, Fig. 18, I have reproduced a sympathetic cell with pericellular plexus, taken from one of my most successful preparations, where the structures other than the spiral fiber and pericellular plexus may be seen. This sketch is made from a section of an inferior cervical ganglion of *Chelydra serpentina*, stained in methylene blue and double-stained in alum carmine. In this sketch the cell body of the sympathetic cell came out more deeply colored than in the section; in all other respects the coloring and arrangement of nerve fibers and nuclei were followed as accurately as possible. In this figure may be seen *B*, the cell body, *C* and *D*, the nucleus and nucleolus, and *A*, the process of a large unipolar sympathetic cell. The capsule, *c*, was clearly made out and could be followed for some distance along the process, when it became unobservable. Three medullated fibers, *a*, *a'*, and *a''*, take part in the formation of the spiral and pericellular plexus. Their interlacement is, however, such that they could be followed for only a short distance. At their distal end these spiral fibers break up into secondary branches, which become very varicose and, after further branching, form a basket-like network which surrounds the cell body of the sympathetic cell, the arrangement of which is better shown in the figure than I can describe it. The figure shows most clearly that this network is intra-capsular. The figure may further serve to give an idea of the number and the arrangement of the intra-capsular nuclei. These, as has been previously observed, are regarded as nuclei of neuroglia cells. Fig. 19 has been added to give the general appearance of a methylene blue preparation of a sympathetic ganglion of a tortoise, double-stained in alum carmine. In this section the processes of the cells were only partly stained, and the fact that this sketch is from a section will explain the abrupt ending of some of the branches. The figure shows three of the more complex pericellular plexuses, *A*, with spiral fibers, and one simpler pericellular plexus, *B*; shows also the arrangement of the medullated and the non-medullated (varicose fibers in the figure) fibers and of the dendrites as seen in sections.

My reasons for assuming that the intra-capsular, pericellular



plexuses above described are the endings in the sympathetic ganglia of Reptilia of cerebro-spinal fibers may briefly be stated as follows :

a) The medullated fibers in the sympathetic ganglia may, from their size and structure, be regarded as fibers of cerebro-spinal origin.

b) In serial sections of well-stained, sympathetic ganglia, single medullated fibers, or small bundles of such, may often be traced from the white rami into the ganglia, where they undergo division, or give off collateral branches, and may now and then, as shown in Pl. IV, Fig. 17, be traced into a spiral fiber and pericellular plexus.

c) The small dorsal sympathetic ganglia of the Reptilia investigated are in close apposition to the spinal ganglia. By removing the bodies of the vertebrae, the spinal cord, with the roots of the spinal nerves, the spinal and sympathetic ganglia may easily be exposed. After exposing the structures as above indicated, I have, in a large number of experiments, divided the roots of the spinal nerves close to the spinal cord, and removed these with the spinal and sympathetic ganglia, with short segments of the nerves uniting the sympathetic ganglion to the sympathetic chain, and with a short portion of the spinal nerve. The tissues so removed were then fixed in ammonium molybdate, imbedded in paraffin, and cut in serial sections. As a rule, in the sections so obtained only a few of the nerve fibers and nerve cells were well stained in methylene blue. In some series, however, the staining was such that small bundles of medullated fibers could be traced from the anterior root into the sympathetic ganglion. The writer (26) has already drawn attention to the fact that in the dorsal spinal ganglia of Reptilia, stained in methylene blue, a small group of multipolar cells was found within the connective tissue capsule of the spinal ganglion. This group of multipolar cells is found on the ventral side of the ganglion, immediately under the capsule of the ganglion, and separated from the sensory cells by a connective tissue septum. Into this group of cells, which, by reason of their shape and structure, are looked upon as sympathetic cells, I have several times been able to trace medullated fibers coming

from the anterior root ; and now and then such a fiber could be traced into a pericellular plexus of the simpler variety.

Such observations, it seems to me, point conclusively to the cerebro-spinal origin of the medullated fibers ending in the ganglion in intra-capsular, pericellular plexuses.

The non-medullated fibers seen in sections of a ganglion wind their course through the ganglion between its constituent cells. Many of these are no doubt the neuraxes of the sympathetic neurons of the ganglion, although it is very seldom that in section such fibers can be traced to a ganglion cell. This may, however, now and then be done, as may be seen from Pl. IV, Fig. 12. Serial sections show further that some non-medullated fibers enter the ganglia through the interganglionic cords. Their mode of ending in the ganglia has not been definitely ascertained, unless possibly that shown in Pl. IV, Fig. 17 (fibers *c*), and described with that figure, may be their mode of ending.

A brief summary of the observations made on the sympathetic ganglia of Reptilia above described may here be given :

1) The sympathetic neurons of Reptilia may be multipolar, bipolar, or unipolar. The first two types need no further mention. The unipolar cells, very characteristic for the sympathetic ganglia of Reptilia, are relatively large, possessing one large process, varying in length and its relation to the cell body of the respective neuron. All these cells have one neuraxis, which near the cell is non-medullated ; whether at some distance from the cell it becomes invested with a sheath of myelin I am unable to say. The neuraxis arises from the cell body or from some one of the dendrites. The number of dendrites varies. The cell body of all sympathetic cells of Reptilia is surrounded by a nucleated capsule.

2) Medullated fibers, no doubt of cerebro-spinal origin, end in the ganglia, either in simple pericellular plexuses surrounding the multipolar or bipolar cells, or in more complex pericellular plexuses with spiral fibers surrounding the cell body of the unipolar cells. These pericellular plexuses are intra-capsular. The medullated fibers branch in the ganglia, and may thus influence a number of sympathetic cells through their several

pericellular plexuses; each branch probably having such an ending.

3) The ending of the non-medullated fibers in the ganglion has not been determined definitely.

#### SYMPATHETIC GANGLIA OF BIRDS.

The observations here recorded were made on half-grown chickens. A 1% to 4% solution of methylene blue in normal salt was injected into the humeral vein (Owen), just above its division over the distal end of the humerus. The methylene blue was allowed to flow into the circulation as quickly as possible; as in birds, a small quantity of the solution causes a stoppage of the heart. At the expiration of thirty to forty minutes the sternum with the attached muscles was removed, and the heart and lungs displaced to one side. In this way the dorsal sympathetic ganglia are readily exposed. In the chicken the dorsal sympathetic ganglia rest on the spinal ganglia, so that a white ramus as such is wanting. The sympathetic ganglia may, therefore, be best removed with the spinal ganglia; this was usually done. The tissues so removed were fixed in ammonium molybdate, the further treatment being as previously indicated. It should be stated at the outstart that the results obtained on the sympathetic ganglia of birds has been far from what might be desired. And this for two reasons:

1) I have found it very difficult to stain these structures at all well in methylene blue. Very generally, all the structures of the ganglion stain a diffuse blue; in which case, sections show scarcely more than may be seen in sections of these ganglia stained with the ordinary stains.

2) The few sections that were well stained would fade in a few days or weeks, no matter what care was taken. In some few instances, however, results which may be compared with those obtained on other vertebrates have been at my disposal.

In the literature I have been able to consult I find no reference to observations made on the sympathetic ganglia of birds with the methylene blue method, although these ganglia have been stained by Cajal (27), Retzius (28), and Lenhossék (29).

Ramon y Cajal's studies on the sympathetic system were published in three papers, which followed each other in close succession. In his second communication, to which I wish here to refer more particularly, he discusses observations made on dove embryos of the fourteenth and sixteenth day, and chick embryos of the seventeenth and eighteenth day. He here states that the sympathetic cells are multipolar, and that all the processes have the character of nervous processes. He describes long and short processes; the latter end free within the ganglion, the former pass beyond the bounds of the ganglion. In his third communication Cajal retracts in part these statements, and states that while the cells are multipolar, the great majority possess one axis-cylinder branch, the others being dendrites. In a brief account of his observations made on the sympathetic ganglia of vertebrates, Retzius refers in a few lines to those made on chick embryos of the fifteenth to the eighteenth day, in which he merely confirms the conclusions ultimately reached by Cajal as above given.

Lenhossék has studied the sympathetic ganglia of embryo chicks of the tenth and fifteenth day; the best results were obtained with the older embryos studied.

In cross-sections of chick embryos of the above age, stained after the double Golgi method, Lenhossék made the following observations:—Both in the cervical and thoracic regions a ramus communicans is wanting; the sympathetic ganglion lies directly on the median side of the ventral spinal nerve. In the thoracic region the spinal nerve divides into a smaller dorsal branch and a relatively larger ramus ventralis, on which the sympathetic ganglion is found. These observations I can to some extent corroborate on the grown chicken, although, as above stated, I find the sympathetic ganglion, or a portion of it, resting on the spinal ganglion. Lenhossék describes multipolar cells, with one neuraxis and several dendrites, five to ten in number. The dendrites are described as quite thick and relatively short, some ending free without any branching.

The neuraxes are described as taking one of three paths:

- 1) Rarely into one of the peripheral branches of the ganglion going to the viscera.











2) In frontal sections of chick embryos of the fifteenth day it was seen that some of the neuraxes passed up or down in the ramus internodalis, and entered one of the contiguous ganglia.

3) Many of the neuraxes take a peripheral course in the ventral branch of the spinal nerve of the segment.

Both Cajal and Lenhossék were able to trace cerebro-spinal nerve fibers into the sympathetic ganglia of the chick. The latter describes these as coming from the ventral ramus of the spinal nerve, as branching in the ganglia, and as terminating in a "free ending." Cajal was able to trace such fibers to the anterior root, while Lenhossék is inclined to regard them as coming from the posterior root. Neither of these investigators describes pericellular plexuses in the sympathetic ganglia of birds.

The results obtained by me with the methylene blue method confirm in many particulars the observations made by Cajal and Lenhossék with the Golgi method. I also find the sympathetic neurons multipolar, with numerous dendrites and one neuraxis. The body of such neurons may be irregularly round or oval, or of a triangular shape. In ganglia stained in methylene blue, and in those so stained and fixed in ammonium molybdate and sectioned, chromophile granules may be seen in the protoplasm of the ganglion cell, if the staining is not too intense or diffuse, as is often the case. These granules are very fine and evenly distributed through the protoplasm, as (Pl. V, Fig. 21) a portion of a section of one of the ganglia of the dorsal sympathetic chain may show.

The dendrites, as Lenhossék has correctly stated, are short and thick and not prone to much branching. They form an interlacing network between the cell bodies of the ganglion cells. As the cell bodies of such cells are surrounded with a nucleated capsule (see *c* of Fig. 22), this dendritic plexus is extra-capsular.

Cajal mentions this arrangement of the dendrites, formed by the "short process," and describes it as pericellular nests—"nido pericellular." He further suggests that through such pericellular nests ganglion cells may be physiologically associated.

Van Gehuchten, Sala, and Dogiel have observed a similar arrangement of the dendrites of the sympathetic cells in the ganglia of Mammalia; they, however, regard the nest-like arrangement as accidental. A study of Pl. V, Fig. 21, may show that the latter interpretation seems the more plausible one, and especially if we take into consideration that such dendrites are extra-capsular. It is further to be remembered that if these dendrites have the power to conduct nervous impulses, as they no doubt may have, they would, reasoning from analogy, conduct toward the cell body—be “cellulipetal”; and I am at present not aware of any instance where a dendrite is stimulated by the cell body or dendrites of another neuron. We may thus assume that the arrangement of the dendritic branches of the sympathetic neurons in the sympathetic ganglia of birds is mainly accidental, depending in a great measure on the relative position of contiguous ganglion cells. Participating in this network between the ganglion cells as above described, are found non-medullated and medullated nerve fibers. The former are, no doubt, in part the neuraxes of the sympathetic cells of the ganglion. In serial sections, however, it may be seen that some of the non-medullated fibers enter the ganglia from without; this, both Cajal and Lenhossék have described. The latter pictures in his article (Fig. 13) a bundle of such fibers entering a ganglion. This figure, as the descriptive text shows, was sketched from a dorsal sympathetic ganglion of a 14-day chick. Lenhossék has this to say concerning these fibers: “Wir haben es hier offenbar mit den Fortsätzen von anderweitig, etwa in den visceralen Ganglien gelegenen sympathischen Zellen zu thun.” He goes on to say that the ending of these fibers is by a simple end brush and not by an end basket; and, to quote again: “Wobei Endäste manchmal auffallende Verdickungen zeigten, an den Stellen, wo sie sich an die Zellen anlegten.” An ending such as here described has not been seen by me. The non-medullated fibers, as far as I have been able to determine, are always extra-capsular, and do not therefore end on the cell body of the sympathetic cells. A few times, however, an ending such as shown in Pl. V, Fig. 20, has been seen by me. In this figure, which is a portion of a section,



of 20  $\mu$  thickness, of one of the dorsal sympathetic ganglia, is shown a sympathetic nerve cell with several dendrites. On one of the branches, *d*, is shown the ending of a non-medullated fiber, *u*; the non-medullated fiber being stained somewhat more deeply than the dendrite. In a careful search of many preparations, only a few such endings have been found; yet in relatively thin sections, studied under  $\frac{1}{12}$ -inch oil immersion, they have now and then been quite clearly made out.

Concerning the medullated fibers found in the sympathetic ganglia of birds, the following observations have been made:

It will be remembered that the sympathetic ganglia of the bird lie on the ventral branch of the spinal nerve and partly on the ventral side of the spinal ganglia, and are removed with these structures. In such preparations, stained in methylene blue and examined before fixing, even under a low power, the multipolar-sympathetic cells may be made out with certainty, and axis-cylinders of medullated fibers may now and then be traced between such multipolar cells. Cajal, it will be remembered, describes such fibers as coming from the anterior roots, while Lenhossék regards them as coming from the posterior roots, as may be gathered from the following statement made by him: "In zwei Fällen, schien es mir, als handelte es sich gerade umgekehrt um Fasern die aus dem Spinalganglion kommen, also um sensible Fasern, doch kann ich dies nicht mit voller Bestimmtheit vertreten." In ganglia removed as above stated, and examined before fixing, and especially in those where only a few nerve fibers were stained, I have several times been able to trace axis-cylinders of medullated fibers ending in the sympathetic ganglia toward the anterior root; this may also be seen in serial sections of the sympathetic ganglia and the structures in connection with them. My observations, as far as they go, are therefore in accord with those given by Cajal.

There is no doubt that many of the medullated fibers ending in the sympathetic ganglia of birds, do so in pericellular plexuses, although Cajal, Retzius, and Lenhossék were unable to see them in Golgi preparations of these structures. The latter says in this connection: "Von einer faserkorbartigen Anordnung im Inneren des sympathischen Ganglion ver-

mochte ich beim Hühnchen nichts wahrzunehmen; es handelte sich immer um einfache Aufzweigungen." The pericellular plexuses seen by me are the endings of collateral branches, or ultimate endings of medullated fibers. A number of such endings are reproduced in Figs. 20-22. In Fig. 20, from the medullated fiber *A*, is given off at the node of Ranvier, *x*, a non-medullated collateral branch, *a*, which may be traced into two pericellular plexuses. Fig. 22 was sketched from a section of a methylene blue stained, dorsal sympathetic ganglion of a chicken, fixed in ammonium molybdate, the sections being further stained in alum carmine. Here the relation of the pericellular plexus to the cell body of the enclosed cell and its capsule is clearly shown.

The pericellular plexuses observed in birds are of a relatively simple structure. The end branch, or collateral branch destined to form such an ending, divides, just before or after it reaches the capsule of a sympathetic cell, into a number of fine varicose fibers which are woven into a very loose plexus and often end free on the cell.

My observations on the sympathetic ganglia of birds may be briefly summarized as follows:

- 1) The sympathetic neurons are multipolar, with one neuraxis and several dendrites. The cell body of such a neuron is enclosed in a nucleated capsule.

- 2) The non-medullated fibers entering the ganglia from without, end, after branching, on the dendritic branches of the sympathetic neurons.

- 3) The medullated fibers of cerebro-spinal origin, which enter the ganglia, most probably from the anterior roots, end, after branching in the ganglia, in pericellular plexuses of a relatively simple structure. These plexuses surround the cell bodies of the sympathetic neurons and are intra-capsular.

#### SYMPATHETIC GANGLIA OF MAMMALIA.

The sympathetic ganglia of Mammalia have been investigated with the Golgi method by Kölliker (30-33), Ramon y Cajal (34-36), Retzius (28), Van Gehuchten (37), Sala (38), d' Erchia

(39), and Lenhossék (29); and with the methylene blue method by Aronson (19) and Dogiel (40, 41). Each writer has in turn reviewed the work of those who have preceded him, to such an extent that a special review of the literature seems here uncalled for. The observations made by others will thus be considered in giving my own results.

The observations here to be recorded cover a period of now nearly three years; in the earlier portion of this work the Golgi method was to some extent used, but in the last two years the *intra-vitam* methylene method alone was used, and the results to be recorded pertain exclusively to observations made with it.

The animals investigated varied in age from such as were two or three weeks old to such as were full grown. The methylene blue solution was injected through the jugular or femoral vein, usually the former; the quantity varying with the size of the animal.

The ganglia studied were the superior and inferior cervical, the stellate ganglion, the smaller ganglia of the chain, and many of the peripheral ganglia. These were exposed forty-five minutes to an hour after the injection; were fixed in ammonium molybdate and studied in sections, either stained only in the methylene blue or double-stained in this dye and alum carmine.

Some of the results here to be given were known to me before Dogiel's (40) article (from which I shall quote freely and to some extent follow) reached me. It seems, nevertheless, advisable to give them, for, notwithstanding the fact that we have both used the methylene blue method in our investigations, Dogiel's observations were made on tissues stained on the slide and fixed in ammonium picrate; and, furthermore, his observations pertain more particularly to the smaller peripheral ganglia (wall of the gall bladder and so forth), where it is possible to study the ganglia as a whole. He was in this way able to obtain preparations of sympathetic neurons, which for completeness of staining, judging from his pictures, seem not to have been equaled. My own observations, as above stated, were made on sections, usually double-stained in alum carmine; and while

in such sections the sympathetic cells are not so clearly shown, by reason of the fact that many of the processes are cut from the cells, yet the relation of the dendrites and pericellular plexuses to the cell body and capsule of the sympathetic cells of the ganglion is more clearly shown than in ganglia studied as a whole.

#### SYMPATHETIC NEURONS OF MAMMALIA.

All writers who have reported observations made with the Golgi or methylene blue method are agreed that the great majority of the sympathetic neurons are multipolar. Dogiel (40) states that near the poles of the sympathetic ganglia bipolar and unipolar cells are to be found. Their number is relatively small. My own observations lead to the conclusion that such cells are usually found between the afferent and efferent nerve fibers of the ganglion. In the protoplasm of all these cells chromophile granules are seen; in this respect my observations are in accord with those made by Dogiel (40).

The nucleus is usually only imperfectly stained in methylene blue, more often of a diffuse blue, which may be of a darker or a lighter hue than the cell body. Nucleoli are only rarely seen with this stain, although they are readily found in preparations stained in alum carmine.

In some Mammalia, namely, the rabbit, hare, and guinea pig, sympathetic cells with two or even three nuclei are found, as has been shown by Remak (44), Guye (42), Schwalbe (15), and more recently Apolant (43). Schwalbe and Apolant explain this curious phenomenon by stating that it is the result of an incomplete cell division, the nucleus dividing but not the protoplasm, and the latter has shown that the multiplication of the nuclei takes place by an amitotic cell division, which may be recognized in embryo rabbits as early as the third week. Apolant reaches the following conclusions concerning this point: "Ich glaube daher, dass die Bildung der beiden Kerne in einem innigen Zusammenhange mit den Wachstumsverhältnissen der Zelle steht, der Art, dass die anfängliche, überwiegende Ausbildung des Kernes zu einer Theilung desselben führt, welche



ihrerseits die Veranlassung zu einem stärkeren Wachsthum der Zelle abgiebt. Ich vindicire also dem Process keine functionelle, sondern lediglich eine biologische Bedeutung für die Zelle." My own observations on sympathetic ganglion cells with more than one nucleus were made on ganglia of the guinea pig stained in methylene blue, a method which, in the hands of Apolant, gave only unsatisfactory results; they may, therefore,



FIG. III. — Sympathetic neurons of guinea pig. (For description see text.)

receive this brief mention. The majority of my preparations were from the solar ganglion. The larger number of the sympathetic neurons in this ganglion are multipolar, with two and sometimes three nuclei. Such cells are reproduced in *A* and *B* of Fig. III; as may be seen from this figure, these cells differ from sympathetic cells found in other Mammalia only in having more than one nucleus. The number of dendrites varies; only one neuraxis is made out (*a*, in figure), if the cells are well stained.

In *D* of Fig. III is shown a multipolar cell with two nuclei, where the portions of the cell body containing the nuclei are united by a band of protoplasm, giving the appearance of a cell in the later stages of cell division. This condition was seen only a few times, and is very much like that shown by Apolant in Figs. 7 and 8 of his article. Not all the multipolar cells have two nuclei, as may be seen in cell *C* of the above figure. Mononuclear multipolar cells are, however, rarely seen. Bipolar cells are proportionally not more numerous in the rodents above mentioned than in other Mammalia. They are found near the poles of the ganglia, between the afferent and efferent nerves, and may be mononuclear or possess two nuclei. In order to close the discussion of these cells I may be allowed to anticipate somewhat, and state that I have often found the multipolar cells, with two or more nuclei, surrounded by a pericellular plexus; one such is shown in *E* of Fig. III.

I must, therefore, agree with Apolant when he states that the presence of two or more nuclei in the sympathetic cells of the rodents under discussion is not to be looked upon as expressing a degenerative process. In all other respects the structure of these cells, — the presence of chromophile granules, — as also the structure of the ganglia taken as a whole, is identical with the structure of the sympathetic cells and ganglia of other mammals, in which the sympathetic neurons have only one nucleus.

*The Dendrites.* — In the sympathetic cell near the center of the ganglion, the dendrites, the number of which varies, may arise from any part of the cell body. In the peripheral cells, as Dogiel (40) has correctly stated, the dendrites are usually given off from that portion of the cell body pointing toward the center of the ganglion. The dendrites branch and rebranch and form between the ganglion cells an intercellular plexus. This plexus is well shown in Fig. 26 — a portion of a section of the solar ganglion of a cat. It may here be seen that the dendrites intertwine in such a way as to leave open spaces, in which the cell body of one, two, or perhaps three sympathetic cells are found. This basket-like arrangement is what Ramon y Cajal has described as pericellular nests. The dendrites do not,



however, come in contact with the cell bodies of the sympathetic cells, but are separated from them by their capsule.

Dogiel (40) has further described a "general peripheral plexus," situated under the fibrous capsule of the ganglion. In the formation of this kind of plexus, dendrites from nearly all the cells of the smaller ganglia and many of the cells of the larger ganglia take part, the dendrites from the cells situated more centrally in the ganglia winding their way out until the plexus is reached.

*Neuraxis.*—It is now very generally conceded that the sympathetic neurons of Mammalia possess one neuraxis — axis-cylinder. This may arise from the cell body or from a dendrite at a variable distance from the cell body. In making the latter statement I have been guided largely by observations made by others, as in sections where, as a rule, only relatively short segments of the axis-cylinder are met with, it is often exceedingly difficult to classify the processes of any particular sympathetic cell. In most preparations the axis-cylinder branches are not stained in any way characteristically. Sometimes, however, as may be seen in Pl. V, Fig. 26, they stain a deeper and more purple shade of blue, which color differentiation may aid in making out which one of the several processes of a sympathetic cell is the axis-cylinder. In such sections I have been able to confirm the statements made above. Near the cell body the neuraxes of the sympathetic cells of Mammalia have a very regular contour and maintain about the same size; and Dogiel (40) has described a very delicate longitudinal striation, caused by a deeper staining of the ultimate fibrillae of the axis-cylinder.

Kölliker (32), who has for many years paid especial attention to the structure of the neuraxes of sympathetic cells, has quite recently summarized his observations as follows:

- 1) The sympathetic nerve fibers (axis-cylinder processes of sympathetic cells) are in many cases surrounded by a very delicate sheath of myelin.
- 2) In some of these fibers the sheath of myelin accompanies the neuraxis to its periphery — nerve fibers from the ciliary ganglion and the pilo-motor nerves of the cat.

3) In other instances the medullary sheath is sooner or later lost, the neuraxes continuing as Remak's fibers — fibers going to the intestine, liver, and spleen.

4) In many cases the neuraxes of sympathetic neurons are non-medullated throughout. This, it would seem to me, is the structure of the neuraxes of sympathetic cells in the peripheral ganglia — those of the heart, salivary glands, intestine, bladder, etc.

Lenhossék (29) and Dogiel (40) have described the giving off of collateral branches from the axis-cylinders of the sympathetic cells of mammalia; such branches have been traced into the intercellular plexus; their mode of ending has not, however, been determined.

Before closing the discussion of the sympathetic neurons in Mammalia, it is necessary to mention some cells found in the sympathetic ganglia, which Dogiel (41) has recently described as sensory sympathetic cells. These cells are said to have the following structural peculiarities: The cells are multipolar, with one to sixteen dendrites and one neuraxis; they are characterized by the structure of the dendrites, which are longer and more slender than the dendrites of the other sympathetic cells. These dendrites ramify in the ganglion and may often be traced into one of the nerve trunks connected with the ganglion, in which they may often be followed for long distances. In the ganglia of Auerbach's plexus such dendritic processes could be followed from a ganglion into one of its nerve roots, and then some into the mucosa, others into the submucosa, and so on. The neuraxes of such cells are described as coming from the cell body or from some dendrite; in the smaller ganglia such cells have non-medullated axis-cylinders; in the larger ganglia of the chain this process becomes surrounded with a medullary sheath some distance from the cell body of the sympathetic cell from which it arises. In the plexuses of the intestine Dogiel was able to trace the neuraxes of such sensory sympathetic cells through several ganglia. In the ganglia through which they pass, or in which they end, collateral branches are given off which terminate in the intercellular plexus. Dogiel suggests that such cells may form the anatomical basis for certain phe-

nomena, such as peripheral reflexes, etc., which have been observed in the sympathetic system. My own observations, based almost entirely on sections, do not give me sufficient evidence on which to judge these interesting and, if corroborated, most important observations which Dogiel has given us. Cells with long, slender processes, such as he has described, have now and then been seen by me, but I have not been able to trace these processes for any distance. I may, therefore, dispense with any further discussion of these so-called sensory sympathetic cells.

*Non-Medullated and Medullated Nerve Fibers in the Sympathetic Ganglia.* — In sections of sympathetic ganglia stained in methylene blue, smaller and larger medullated fibers and non-medullated fibers may be seen between the ganglion cells. These have been observed by Kölliker, Ramon y Cajal, Lenhossék, Sala, Van Gehuchten, and others, in Golgi preparations of the sympathetic ganglia of Mammalia, and by Aronson and Dogiel in methylene blue stained preparations of these structures. In serial sections of the chain ganglia removed with the white rami, stained in methylene blue, bundles of medullated fibers may readily be traced from the white rami into the ganglia, although it is not always easy to trace individual fibers for any long distance. In the ganglia such medullated fibers are seen branching into two or three branches, which may or may not be medullated, and in a number of instances such branches were traced to a sympathetic cell, where, after further branching, they terminated in a pericellular plexus which surrounded the cell body of the sympathetic cell. In well-stained preparations such pericellular plexuses are easily found, although in sections it is not always easy to connect such plexuses with any particular nerve fiber. These pericellular plexuses vary much in complexity and in the arrangement of fibrils which form them. They may be very loosely woven, or, again, made up of a large number of fibrils. The fibrils may be quite smooth, or show numerous and large varicose enlargements. The ones shown in Pl. V, Fig. 23, a small portion of a section of the stellate ganglion of a dog, stained in methylene blue and alum carmine, may be looked upon as presenting the general appear-

ance of the pericellular plexuses in the sympathetic ganglia as seen by me. In double-stained sections there can be no doubt that the pericellular plexuses are in contact with the cell body of the sympathetic cell—are intra-capsular. This agrees with the following statement found in Dogiel's (40) account: "Das-selbe" (speaking of the pericellular plexuses) "liegt unmittelbar der Oberfläche der Ganglienzellen an und befindet sich, wie mir scheint, zum Unterschiede von dem intercellularen Geflecht nicht über, sondern unter der Zellenhülle." The fibers terminating in the pericellular plexuses are usually non-medullated for some distance from the cells around which they end (*a*, Pl. V, Fig. 23), although in a few instances they are medullated to the point where they pierce the capsule, *b*, of the same figure. In some few instances much more complicated pericellular plexuses were seen, in which the fiber or fibers terminating in such plexuses were spirally wound around some process, probably the axis-cylinder branch of the cell enclosed by the pericellular plexus. One such ending is shown in Pl. V, Fig. 24, taken from the stellate ganglion of a dog. In the few instances seen by me the network resulting from the division and redivision of such spiral fibers is much more complicated than the pericellular plexuses usually observed. The fibrils are very varicose, often presenting quite large nodular enlargements. Aronson (19) has described spiral fibers in the sympathetic ganglia of the rabbit. His description leads one to infer that they are quite common. A comparison of the figures given by Aronson (especially Fig. 1) with my Pl. V, Fig. 24, may suffice to show that very dissimilar structures are spoken of in the two accounts. Spiral fibers, such as he describes, formed by one, two, or three varicose fibers, twisted once, twice, or three times around the neuraxis of the sympathetic cell and terminating in an ordinary pericellular plexus, have now and then been seen by me. In this account, however, the term "spiral fiber" is confined to such as are diagrammed in Pl. V, Fig. 24, ending in a more complex pericellular plexus. These were only rarely seen by me. They resemble, to some extent, the more complicated pericellular plexuses, with spiral fibers, described for Reptilia.











Pericellular plexuses, enclosing the cell body of the sympathetic neurons of Mammalia, have been described for nearly all sympathetic ganglia in all parts of the sympathetic system; in the cranial sympathetic ganglia as follows: Ciliary ganglia by Kölliker (32) and Michel; sphenopalatine by Lenhossék (29); and submaxillary and sublingual by myself (45); in the larger ganglia of the chain by a number of investigators, beginning with Aronson (19); in the respiratory passages by Arnstein (46); in the heart of the rabbit by Aronson (19); intestinal canal and other peripheral ganglia by Dogiel; in the suprarenal by Dogiel (47); in the epididymis by Timofeev (48); and have further been seen by me in the bladder and oesophagus of the cat. In all the Mammalia studied, and in all the ganglia, they have essentially the same structure, and so far as my observations go, in sections of the ganglia of the sympathetic chain and various peripheral ganglia of the dog, cat, rabbit, and guinea pig, the pericellular plexuses are always intra-capsular. Sometimes only one fiber may end in a pericellular plexus; sometimes two, three, or even more fibers may take part in the formation of one plexus; these fibers are always, so far as may be gathered from the investigations on the sympathetic ganglia of Mammalia, collateral or end branches of medullated fibers.

So far as concerns the small medullated fibers, which may be traced from this or that nerve root of a ganglion into said ganglion, my own observations confirm wholly the account given by Dogiel (40). Such fibers may readily be seen in sections of methylene blue stained ganglia, where they are found branching and rebranching, and forming, with the dendritic processes of the ganglion cells, what Dogiel has described as the intercellular plexus; this plexus is always extra-capsular and may be seen in Pl. V, Fig. 26—a portion of a section about  $20\mu$  in thickness. In sections one-third or one-half that thickness I have now and then observed what I have looked upon as the ending of the fibers under discussion. In Pl. V, Fig. 25, is reproduced a cell from a section about  $10\mu$  in thickness of the solar ganglion of a cat stained in methylene blue. The cell body of this cell was deeply stained, its neuraxis, *a*, and dendrite, *b*, not so deeply; these could, however, be clearly

made out. A very small non-medullated fiber, *c*, could be traced with the utmost clearness to its ending on one of the protoplasmic branches of the cell in question; this non-medullated fiber terminated in two very small nodular enlargements, which were in contact with this dendrite, as shown in *c'* of this figure. In a number of instances such endings were made out, so that I feel justified in concluding that at least some of the non-medullated fibers in the ganglia, possibly also the terminal branches of the *small* medullated fibers, terminate in the ganglia after this manner.

In giving my own conclusions as to the nature of the fibers ending in the sympathetic ganglia of Mammalia, I can do no better than to quote the conclusion reached by Dogiel (40) concerning this point, which is as follows: "Die feinen Fasern, welche in den Ganglien mit intercellularem Geflechte endigen, zu den sympathischen, augenscheinlich vorzugsweise markhaltigen Fasern gehören, die dicken Fasern aber, deren Endverzweigungen in den Ganglien pericellulare Geflechte bilden, zu den markhaltigen Fasern zu rechnen sind, welche aus dem Cerebrospinalsystem entspringen." These pericellular plexuses, as already stated, are always intra-capsular.

#### GENERAL CONCLUSIONS.

In these general conclusions my aim is to be as brief as possible. Two reasons may here be given in justification of this: (1) the results obtained in each of the vertebrate classes studied have to some extent been summarized in the foregoing pages; (2) the writer has, in a series of "Special Lectures on the Sympathetic Nervous System," given before the medical students of Michigan University, and published in the *Journal of Comparative Neurology*, Vol. VII, No. 2, September, 1897, dwelt more fully on many of the questions which will here be touched upon.

From a study of the sympathetic ganglia of vertebrates the following facts concerning the shape and structure of the sympathetic neurons, and the nerves ending in the ganglia, may be deduced:



1) In all vertebrates, excepting the Amphibia, the great majority of the sympathetic neurons are multipolar, possessing a varying number of dendrites; but each cell has only one neuraxis. In Amphibia the sympathetic neurons, with the exception of those found in the coats of the intestinal canal and stomach, which are multipolar, are unipolar cells, as Kölliker has previously stated. In vertebrates other than Amphibia, some few unipolar and bipolar sympathetic neurons are to be found.

2) In all the vertebrates examined chromophile granules were found. One relatively large nucleus is the very general rule; the sympathetic cells of rabbits, hares, and guinea pigs forming an exception; many sympathetic cells with two or, occasionally, even three nuclei being here found.

3) The neuraxes of sympathetic cells may be medullated throughout, medullated for only a portion of the process, or non-medullated throughout; the medullary sheath, if present, forms a relatively thin layer, thinner than in the small medullated cerebro-spinal fibers. (The above statements are taken from Kölliker's writings.)

The neuraxes of sympathetic neurons have one or the other of the following distributions:

- a) To involuntary smooth muscle or heart muscle.
- b) To glandular tissues.
- c) To other sympathetic ganglia (?).
- d) To the spinal ganglia.

In the special lectures above alluded to, the writer has discussed, somewhat at length, each of the above possible modes of termination of the neuraxes of sympathetic neurons, considering also the literature bearing on this subject. It may here suffice to add that Arnstein (46) has recently traced, and pictured the neuraxis of a sympathetic neuron ending in smooth muscle tissue; the writer has traced small branches of non-medullated fibers from some of the small ganglia found in the cat's auricle to their ending on heart muscle, and also the neuraxes of sympathetic neurons of the sublingual ganglion (Langley) to the epilamellar plexus surrounding alveoli of the gland of the same name; from Dogiel's (40) work, some of

which I have corroborated, the conclusion may be drawn that neuraxes of sympathetic neurons (especially those surrounded by a thin layer of myelin) end in the intercellular plexuses of sympathetic ganglia, probably on the dendrites of sympathetic cells; and, finally, Ramon y Cajal's (36) and Dogiel's (49) observations on the ending of sympathetic nerve fibers in the spinal ganglia of Mammalia support the view that the neuraxes of sympathetic neurons end in the spinal ganglia.

4) The cell bodies of sympathetic neurons are surrounded by a nucleated capsule, which apparently has the same structure in all vertebrates.

5) In the sympathetic ganglia of all vertebrates studied by others and myself (speaking here of results obtained with either the Golgi or the methylene blue method), medullated fibers ending in pericellular intra-capsular plexuses have been found. These are, in all instances described, of essentially the same structure. I am well aware, as the accompanying plates may show, that the relation of the pericellular plexuses to the cell bodies of the enclosed sympathetic neurons varies somewhat in the different vertebrates, as do also the course, structure, and relation to other processes of the nerve fibers ending in such pericellular plexuses; yet these differences are not essential and important. The question now arises, What is the origin of the medullated fibers thus ending in the pericellular plexuses? "Bei der Ermittlung der schwierigen, hier zur Sprache kommenden Verhältnisse haben sich" (as Kölliker aptly states) "die Physiologie und die feine Anatomie brüderlich die Hand gereicht." The anatomical side of the question has been repeatedly touched in the preceding pages, where it may have been seen that abundant observations have been made, both with the Golgi and methylene blue method, to show that such medullated fibers enter the ganglion either through the white rami (chain ganglia) or through some nerve root of the ganglion. Once in the ganglion, the medullated fibers have been shown to branch and even rebranch before ending in the pericellular plexus.

Physiologists have long known that all sympathetic effects, which may be produced by stimulating a sympathetic nerve in any region, may also be produced by stimulating some spinal

nerve within the vertebral canal. This, as is now well known, and first clearly shown by Gaskell (50), is explained by the fact that certain small medullated fibers, which leave the cord through the anterior roots of certain spinal nerves — first dorsal to the third or fourth lumbar inclusive — reach the sympathetic ganglia through the white rami communicantes. These fibers end, not only in the ganglia of the chain, but also may be traced into the pre-vertebral and even the peripheral ganglia, as the following statement taken from Gaskell's account may show:

“The white rami communicantes are formed by an outflow of medullated fibers from both the anterior and posterior roots of the spinal nerves between the second thoracic and second lumbar inclusive, which medullated fibers pass not alone into their metameric sympathetic lateral ganglia, but also form three main streams, upwards into the cervical ganglia, downwards into the lumbar and sacral ganglia, and outwards into the collateral (pre-vertebral) ganglia. The white rami communicantes alone constitute the rami viscerales of the morphologist. The outflow of visceral nerves from the central nervous system into the so-called sympathetic system takes place by their means alone.”

That these small medullated fibers are the fibers which, in all vertebrates studied, end in the sympathetic ganglia in pericellular plexuses, may now be assumed with much certainty; and this for two main reasons:

a) Medullated fibers have been traced from the white rami into the sympathetic ganglia, or from some nerve root into the peripheral ganglia, and have been seen to end in pericellular plexuses, as repeatedly stated.

b) The researches of Langley (51), Anderson, and Dickinson have taught us that in nicotin we have a drug which shows most clearly that the action of such fibers is interrupted in the sympathetic ganglia; that the sympathetic effects which may be obtained on stimulating a cerebro-spinal nerve, containing the small medullated fibers first described by Gaskell, or a white ramus made up of such fibers, cannot be obtained if a solution of nicotin of proper strength be injected into the circulation of the animal or be applied to the sympathetic ganglia with which such fibers are connected.

Such observations led Langley to conclude that the nerve fibers, issuing from the cord and reaching the sympathetic ganglia through the white rami, had each a sympathetic nerve cell in its course, which sympathetic cell was paralyzed by nicotin. Langley has suggested the term pre-ganglionic fibers or pre-cellular fibers to designate the efferent medullated fibers before they reach the nerve cells, and post-ganglionic or post-cellular fibers after they have traversed the nerve cells. Taking these statements into consideration, it would seem reasonable, to say the least, that the pre-ganglionic fibers are the medullated fibers which end in the sympathetic ganglia in pericellular plexuses, and the post-ganglionic fibers, the neuraxes of the sympathetic neurons of said ganglia.

It is not, however, to be supposed that such pre-ganglionic fibers end only in the chain ganglia in pericellular plexuses. Small medullated fibers may, as we have quoted from Gaskell, be traced into the peripheral ganglia, and the mere fact that in such ganglia pericellular plexuses, similar in structure to those found in the chain ganglia, have been described as the endings of medullated fibers, would warrant the conclusion that many of the pre-ganglionic fibers reach also the peripheral ganglia. Kölliker (33) has stated the fact so tersely that I may be allowed to quote again from him: "Hierbei ist der Verlauf derselben" (meaning here the pre-ganglionic fibers) "ein längerer oder kürzerer. Die einen enden an den nächstgelegenen Ganglienzellen, andere durchlaufen mehrere Ganglien, bevor sie zu ihren Endigungen gelangen und können hierbei durch Collateralen auf eine Mehrheit von Zellen einwirken. Noch andere endlich finden erst an den am meist peripherisch gelegenen Ganglien ihr Ende, wobei es unentschieden bleibt, ob sie in ihrem Verlaufe auf zwischengelegene Zellen einwirken."

These observations warrant, it seems to the writer, the following statements: the sympathetic neurons form the peripheral links in a nerve chain, of which the second link is formed by a neuron, the cell body of which is situated in the cerebro-spinal axis, the neuraxes of which form the pre-ganglionic fibers ending in pericellular plexuses, which unite the two links physiologically.



It is probable, then, that in all vertebrates this statement holds good: an impulse, leaving the cerebro-spinal system, and having sympathetic effects, is transferred from a pre-ganglionic fiber to one or several sympathetic cells which convey it along their neuraxes to the periphery.

The writer would here again offer the suggestion first made in the lectures above referred to, that nicotin does not primarily paralyze the sympathetic cells of the sympathetic ganglia, but the pericellular plexuses, the endings of the pre-ganglionic fibers in the sympathetic ganglia. This theory, for it is but a theory, is based on the analogy which exists between the physiological action of nicotin and certain other drugs, notably curare. The latter drug, as is well known, paralyzes the motor endings in striped muscle, and has an action very similar to nicotin on the sympathetic ganglia; on the other hand, nicotin paralyzes also the motor endings in striated muscle, not quite so readily as curare, but in a similar manner; its action on the sympathetic ganglia has already been explained. It would seem reasonable, therefore, to suppose that, in both cases above alluded to, curare and nicotin paralyze the ending of the cerebro-spinal fiber; in the one case, the motor ending in striped muscle; in the other case, the pericellular plexuses in the sympathetic ganglia.<sup>1</sup>

The question as to whether the neuraxes of sympathetic cells may end in other sympathetic ganglia, and may in this way influence other sympathetic cells, seems as yet open to discussion. Kölliker and Langley are of the opinion that the neuraxes of sympathetic neurons end always in the periphery, in involuntary muscle, gland tissue, etc.; while Dogiel, with whom my own observations on this point lead me to concur, believes that the fine fibers, which end in, and help to form the intercellular plexus of the sympathetic ganglia, are the neuraxes of sympathetic neurons, more especially the myelinate ones. They end, I believe, on the dendrites of the sympathetic neurons of the ganglion.

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July, 1897.

<sup>1</sup> In his later writings, Professor Langley gives this explanation of the action of nicotin on the sympathetic ganglia.



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NOTE.—In a number of instances the reference number is repeated, as the same thought is expressed in two or more articles by the writers mentioned.

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The literature which has appeared since this article was sent to the editor will receive consideration in a future publication. — G. C. H.



## EXPLANATION OF PLATE III.

All figures, with the exception of Nos. 1, 19, and 26, were sketched with the aid of camera lucida under a  $\frac{1}{2}$ -inch oil immersion and No. 1 eyepiece, the image being reflected to the table, this giving a magnification of 890 diameters. Figs. 1, 19, and 26 were sketched under a No. 7 objective and No. 1 eyepiece, with about 400 diameters amplification.

FIGS. 1-7. *Sympathetic Ganglia of Fishes.*

FIG. 1. Sympathetic neurons from sections of sympathetic ganglia, stained in methylene blue, of small-mouth black bass (*Micropterus dolomieu* Raf.); *a*, *b*, and *c*, unipolar cells; *d* and *e*, multipolar cells.

FIGS. 2, 3. Pericellular plexuses found in sections of sympathetic ganglia of black bass. Methylene blue stain. *a*, neuraxis, ending in pericellular plexus. In Fig. 3 the enclosed ganglion cell is faintly indicated in black.

FIG. 4. From teased preparation of sympathetic ganglion of black bass, stained in methylene blue, fixed in ammonium molybdate and hardened in alcohol. *c*, sympathetic cell; *n*, nucleus; and *c*, its capsule; *a*, medullated fiber terminating within the capsule in pericellular plexus.

FIG. 5. The same cell, double-stained in alum carmine. Some of the end branches of pericellular plexus terminate between the intra-capsular nuclei.

FIG. 6. Completely isolated cell, obtained by teasing a sympathetic ganglion of black bass, stained in methylene blue and alum carmine. *a*, cell body of a sympathetic neuron; *b*, medullated fiber ending within capsule *c*.

FIG. 7. From the same ganglion from which Fig. 6 was taken. *A*, large medullated fiber giving off two collateral branches, *a'* and *a''*, ending within the capsules of cells *A* and *B*, respectively.

FIGS. 8-11. *Sympathetic Ganglia of Amphibia.*

FIG. 8. Small portion of a section of sympathetic ganglion of *Rana Catesbiana*, stained in methylene blue; only spiral fibers and pericellular plexuses stained. Shows the connection of spiral fiber, *a*, with pericellular plexus, the structure of which is well shown in this figure. In *d* we may see one of the large nodular swellings now and then seen; *e*, free ending of fibril of the pericellular plexus.

FIGS. 9, 10. Two ganglion cells from section of sympathetic ganglion of *Rana C.*, stained in methylene blue and alum carmine. *a*, neuraxis of unipolar cell; *b*, sheath nuclei of the neuraxis; *c*, capsule; *d*, nodular swellings in pericellular plexus; *e*, free endings of fibrils of plexus; *s. f.*, spiral fibers.

FIG. 11. Sympathetic neurons of Auerbach's plexus of large intestine of *Rana C.*, stained in methylene blue and fixed in ammonium picrate. *a*, neuraxis; *b*, dendrites.

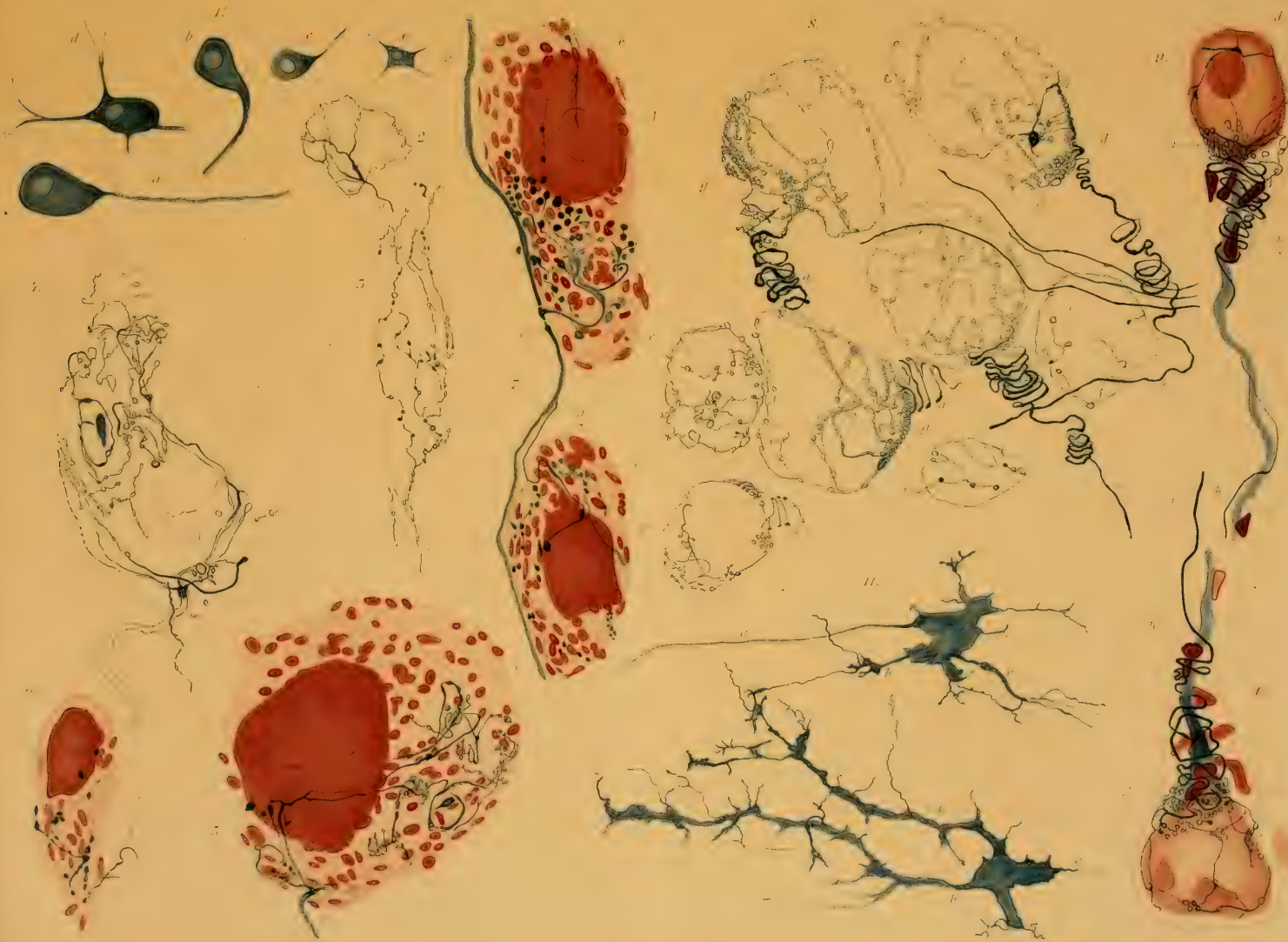


















## EXPLANATION OF PLATE IV.

FIGS. 12-19. *Sympathetic Cells of Reptilia.*

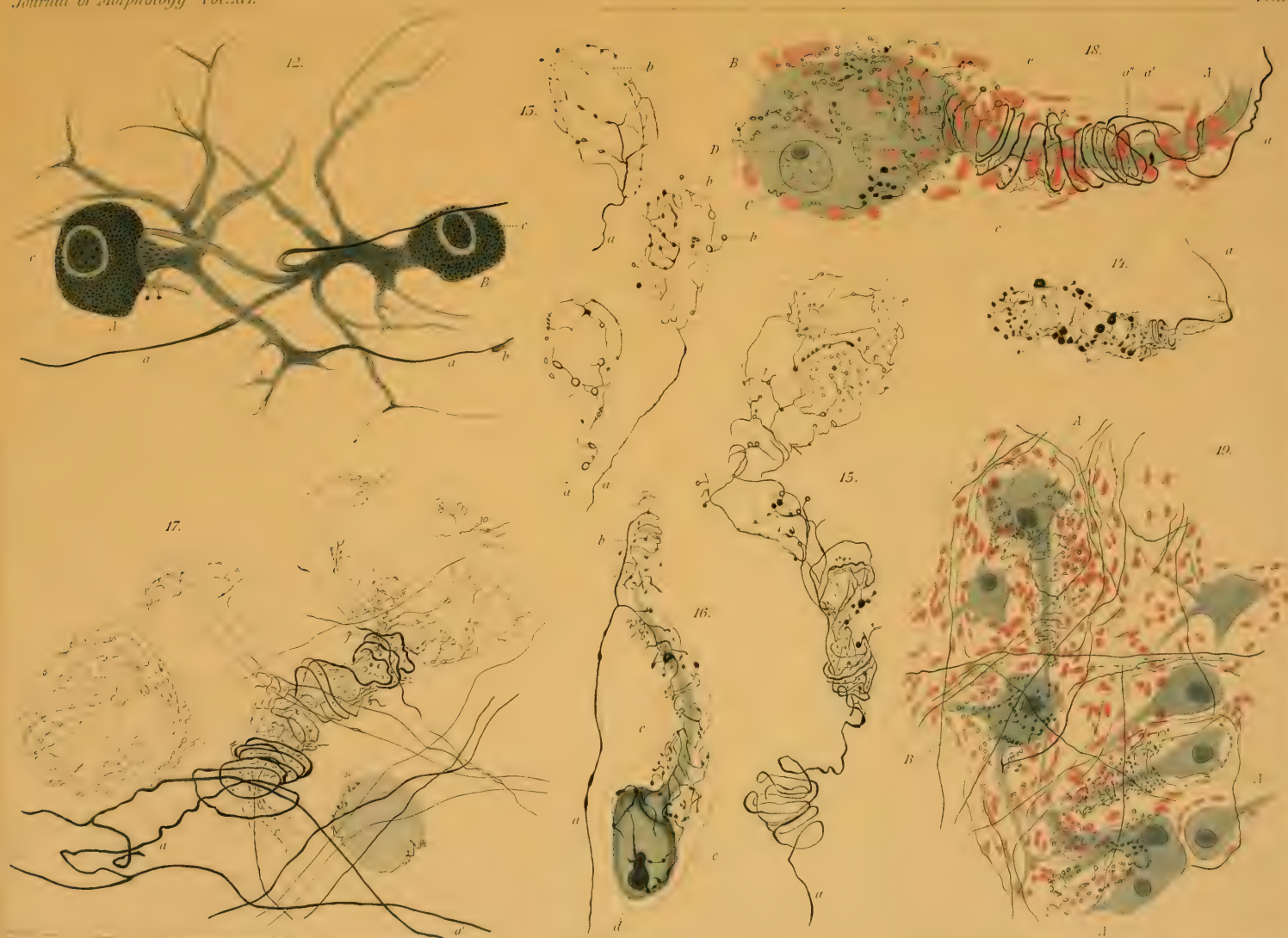
FIG. 12. Two sympathetic cells from the inferior cervical ganglion of *Chrysemys picta*; methylene blue stain. *a*, neuraxis; *b*, sheath nucleus of neuraxis; *c*, clear zone of protoplasm surrounding nucleus; protoplasm shows chromophile granules.

FIG. 13. From section of dorsal ganglion of *Chelydra serpentina*, stained in methylene blue. Simple pericellular plexuses. *a*, neuraxis of fiber ending in pericellular plexus; *b*, free endings of fibrils.

FIGS. 14-17. More complicated pericellular plexuses, with spiral fiber. All sketched from sympathetic ganglia, *Chelydra serpentina*, stained in methylene blue.

FIG. 18. From section of sympathetic ganglion of *Chelydra serpentina*, stained in methylene blue and alum carmine. *A*, neuraxis; *B*, cell body; *C*, nucleus; and *D*, nucleolus of large unipolar sympathetic neuron. *a*, *a'*, *a''*, three neuraxes of medullated nerve fibers, forming a spiral and ending in pericellular plexus; *c*, capsule.

FIG. 19. A portion of a section of an inferior cervical ganglion of *Chelydra serpentina*, stained in methylene blue and alum carmine; showing a number of more complicated pericellular plexuses with spiral fibers, *A*, and one simpler plexus without spiral fiber, *B*. Also the network of medullated and non-medullated fibers between ganglion cells.























## EXPLANATION OF PLATE V.

FIGS. 20-22. *Sympathetic Ganglia of Birds.*

FIG. 20. From methylene blue stained section of one of the dorsal sympathetic ganglia of chicken. *A*, medullated fiber, with node of Ranvier at *x*, at which place is given off a non-medullated collateral branch, *a*, which terminates in two pericellular plexuses; *n*, a non-medullated, probably a sympathetic nerve, ending on *d*, the dendrite of the sympathetic cell.

FIG. 21. A portion of a section of a dorsal sympathetic ganglion of chicken, given to show the arrangement of the multipolar cells and the "intercellular plexus," formed by the dendrites, medullated and non-medullated fibers of the ganglion; *b*, pericellular plexus. The capsules of the sympathetic cells were not stained, and therefore not shown in the figure.

FIG. 22. Three sympathetic cells, with pericellular plexuses from dorsal ganglion of chicken. Methylene blue and alum carmine. *a*, neuraxis of nerve fiber ending in pericellular plexus; *c*, capsules of sympathetic cells.

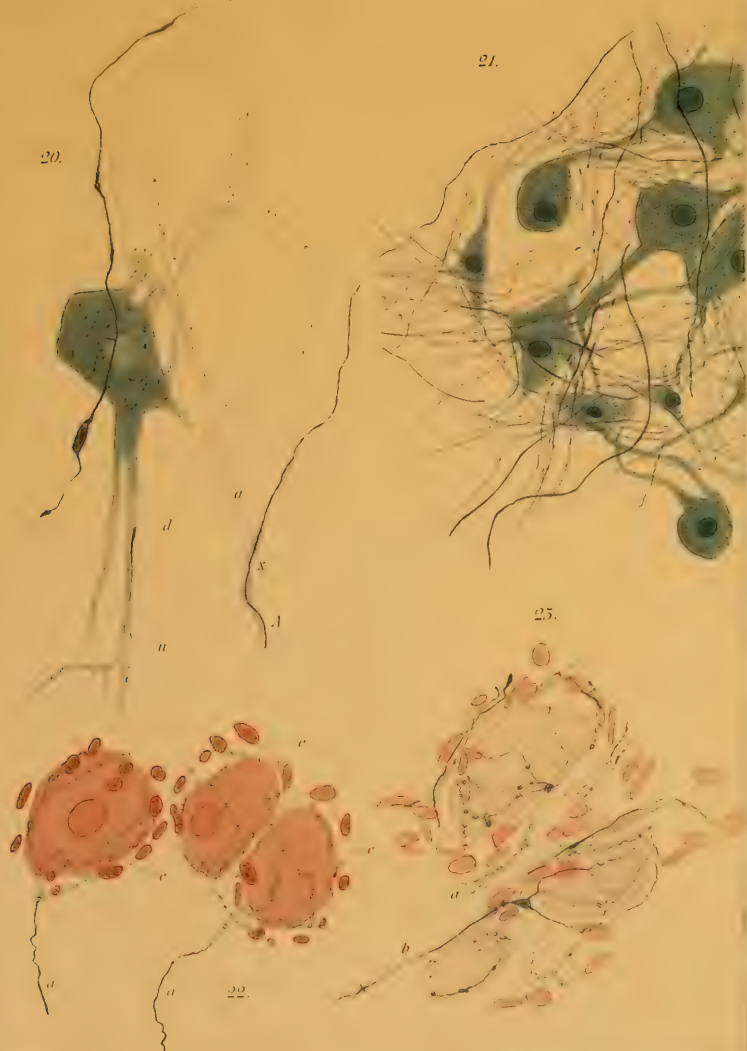
FIGS. 23-26. *Sympathetic Ganglia of Mammalia.*

FIG. 23. A portion of the stellate ganglion of dog. Methylene blue and alum carmine. *a*, non-medullated fiber, collateral branch of pre-ganglionic fiber ending in pericellular plexus; *b*, fiber ending in pericellular plexus which is medullated to within a short distance of the capsule.

FIG. 24. Sympathetic cell from stellate ganglion of dog (methylene blue and alum carmine), showing a more complex pericellular plexus with spiral fibers.

FIG. 25. Sympathetic neuron from semilunar ganglion of the cat, methylene blue staining. *a*, neuraxis; *d*, dendrite; *c*, non-medullated, probably sympathetic fiber ending on dendrite at *c*'.

FIG. 26. A portion of a section of semilunar ganglion of cat, methylene blue staining, showing arrangement of sympathetic cells and "intercellular network" formed by the dendrites of the sympathetic cells and the medullated and non-medullated fibers of the ganglion.



















# STUDIES ON LIMULUS.

## II. THE NERVOUS SYSTEM OF LIMULUS POLYPHEMUS, WITH OBSERVATIONS UPON THE GENERAL ANATOMY.

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## INTRODUCTION.

THE senior author first began work on the nervous system of *Limulus* in 1889, and after various interruptions the distribution of the more important nerves was worked out and recorded in the shape of rough sketches and notes. Especial attention was given to the distribution of the haemal, cardiac, and sympathetic nerves, with the hope of finding facts that might point toward conditions similar to those in vertebrates. In some respects these hopes were not realized, or at least not in the particular ways looked for, while in others, conditions were found that more than exceeded our expectations. For example, a very careful search was made for lateral line nerves similar to those in vertebrates. Various embryological data seemed to indicate that one might be found running parallel with and close to the free edge of the carapace and abdomen, because in the embryos this area is early marked out by a thickened band of ectoderm along which are scattered a series of minute sense organs. Every attempt to find such a nerve in that region both in the embryos and in the adult failed. But a nerve was finally found on the neural surface nearer the median line that fills some of the requirements of a lateral line nerve (Pl. VI, *ln*), for it is a purely sensory cranial nerve extending at right angles to the course of the other cranial and spinal nerves, nearly, if not quite, the whole length of the head and trunk. It branches more freely in the abdominal region and supplies the skin along its course, but the exact nature of the sense organs to which its branches are distributed, and whether this nerve is split off originally from the sensory thickening that runs round the embryo, and which corresponds in position with the edge of the future carapace and abdomen, could not be determined. The fact that it probably does not supply the line of marginal sense organs in the



adult is against such a view of its origin. In another paper we shall describe the central terminations of this nerve and we will then discuss its probable relations to corresponding cranial nerves in vertebrates.

I had hoped to find some points of comparison between vertebrates and *Limulus* in the mode of union of the cardiac nerves with the central nervous system, especially in the relations of the cardiac branches to those cranial nerves arising from what I have called the vagus region of the brain. These inquiries at once led to the discovery of the three great longitudinal cardiac nerves, the nerve plexuses uniting them with one another, and the segmentally arranged nerves by which the cardiac plexus is united with the central nervous system. When we came to study these nerves more carefully we found that we had obtained data showing in *Limulus* an approach toward the well-known conditions in vertebrates. This resemblance is shown: (1) by the absence of cardiac nerves in front of the sixth thoracic neuromere; (2) by the increased size, and (3) by the fusion of the branches arising from the nerves of the vagus region. In the transition from invertebrates to vertebrates, we may assume that as the elongated arachnid heart was forced forward into the head region it gradually lost its connection with the spinal nerves and retained its union with the nerves of the vagus region only. In *Limulus* the fusion with each other of at least two cardiac nerves in the vagus region, and their increased size, indicate the adaptability of these nerves for this purpose.

The main sympathetic system of *Limulus* also presents striking resemblances to the condition found in primitive vertebrates. Instead of the single trunk described by Milne-Edwards, we find two longitudinal trunks, suggesting the condition found in some Amphibia. One trunk is mainly related to the cardiac nerves (Pl. IX, Fig. 6), the other is deeper and nearer the median line and more intimately related to the intestine (Pl. VIII, Fig. 4). Both sets of nerves form anastomosing plexuses. They are united by regularly arranged rami communicantes with the haemal nerves, give off segmental branches to the heart and intestine, and in addition supply

adjacent trunk muscles. The segmental intestinal nerves, like the cardiac nerves, are absent from the anterior five thoracic segments; therefore the intestinal and the cardiac branches of the sixth, seventh, and eighth nerves having a greater territory to cover are larger and better developed than the corresponding nerves in the trunk region. These enlarged intestinal nerves supply that part of the alimentary canal confined to the thorax, exclusive of the oesophagus and proventriculus, into which the liver ducts open. This part corresponds roughly to the gastric region of vertebrates, and the nerves that supply it may thus be fairly regarded as comparable with the intestinal branches of the vagus, especially since they have their origin in spinal nerves which for other reasons we had regarded as comparable with the system of vagus nerves in vertebrates.

We thus have in *Limulus* a complex condition that clearly indicates the initial differentiation of sympathetic and vagus systems similar to those found in vertebrates. This is shown (1) by the presence in *Limulus* of double longitudinal nerve trunks that form anastomosing plexuses, united on one side by segmental rami communicantes with the spinal nerves, and on the other by segmental cardiac and intestinal nerves with the heart and intestine; (2) by the absence or great reduction of segmental cardiac and intestinal nerves anterior to the sixth thoracic neuromere; (3) by the consequent increase in size, and in the territory covered by the intestinal and cardiac nerves of the sixth, seventh, and eighth neuromeres; (4) by the origin of the enlarged cardiac and intestinal nerves of the seventh and eighth thoracic segments from those spinal nerves which on entirely independent grounds we had long ago regarded as comparable with the vagus group in vertebrates.

The sympathetic system of *Limulus* differs from that of vertebrates in the absence of segmental ganglia, although isolated ganglion cells are scattered here and there along the main intestinal and cardiac nerves and over the surface of the intestine.

The division of labor between Mr. Redenbaugh and myself in the preparation of this paper was rather unusual. I began











work on the peripheral nervous system of *Limulus* more than ten years ago, and have continued my observations on it from time to time ever since.

I had thus worked out the semi-maceration method of dissection, the structure of the large, median cardiac valve, the cardiac plexuses, and the distribution of the main peripheral nerves, neural and haemal. A great deal of time was devoted to the distribution of the cardiac nerves, in order to determine their mode of union with the central nervous system; also much time was given to studying out the relations of the longitudinal, sympathetic, and intestinal nerves. Many of the results thus obtained were recorded in the shape of notes and rough drawings, and the entire work was well in hand when it was turned over to Mr. Redenbaugh for completion. By his very careful work Mr. Redenbaugh was able to add many new and important details, especially in regard to those relations of the cardiac and intestinal branches that require such careful dissection. All these points have been verified by both of us, in some cases several times.

The drawings were made in most instances by Mr. Redenbaugh along the lines of my original plans and sketches, but I have added some details of color and finish where it seemed advisable to make them more distinct or more intelligible.

The descriptive parts were written entirely by Mr. Redenbaugh and are presented in very nearly the form accepted by the Biological Department of Dartmouth College as a thesis for the degree of Doctor of Philosophy.

W. PATTEN.

Most of the work in this paper has been done in the Biological Laboratory of Dartmouth College. We wish to acknowledge the kindness of Commissioner J. J. Brice, and of the late Col. Marshall MacDonald, for facilities afforded by the United States Fish Commission Laboratory at Woods Holl, Mass., during the summers of 1894 and 1896.

## 1. HISTORICAL.

Before the year 1872 we find little mention of the nervous system of *Limulus*, although a number of papers had appeared upon the natural history, histology, and systematic position of the King Crab. Van der Hoeven, in 1838, published a monograph upon the anatomy of *Limulus*, in which he gave a very good account of the external form, appendages, and grosser internal anatomy. In the same year Milne-Edwards made the first observations upon the development. Gegenbaur, in '58, described the histology of some of the tissues of *Limulus*. Lockwood, in '70, Dohrn, in '71, and Packard in '72, contributed considerable to our knowledge of the embryology of the animal.

The first important description of the nervous system appeared in a paper by Owen in '72. He figured the brain and ventral cord, and the principal nerves arising therefrom.

Milne-Edwards, in '73, carried the investigation of the nervous system much further, and also gave a very complete account of the circulatory system.

In '80 Packard described the histology of the digestive system, structure of the liver, nephridia and eyes, and gave some observations upon the brain, particularly its internal structure and development. In '93 he published further observations upon the brain with notes upon its embryology. In this paper he deals almost entirely with the internal structure.

Lankester, in '84, described the skeleto-trophic tissues and coxal glands, and in '85, with the assistance of W. B. S. Benham, the muscular and endoskeletal systems.

In '89 Patten gave a short account of the development of the brain, and in '93, treated of it in greater detail, tracing the later modifications to practically the adult stage.

In the year '93 Viallanes, also, published a paper upon the brain of *Limulus*; and Miss Ida H. Hyde investigated the nervous mechanism of the respiratory movements of *Limulus*, and maintained that the respiratory centers were located in the ventral cord.

## 2. METHODS.

The results obtained in our work upon *Limulus* have been obtained largely by careful dissection. In order to accomplish much by this method, however, it has been necessary to prepare the material in a special way. In fresh specimens the nerves were found to be so transparent, and the other tissues so tough, that it was impossible to trace out the smaller nerves with any degree of accuracy, and in most alcoholic material the clotted blood and organic precipitates upon the tissues rendered it difficult to distinguish the smaller nerves from arteries.

Specimens which had been for a long time (two or three years) in alcohol of from 50 per cent to 70 per cent were found to be in remarkably good condition for dissection. The nerves were white, and easily traced in the partially macerated tissues when dissection was carried on under water or weak alcohol.

Equally good material was procured by taking large female *Limuli* at the end of the spawning season, when all the ova had been shed, and treating them in the following manner. They were first allowed to bleed freely, then cut in halves along the median line, and the parts macerated for several days in water. Finally, they were transferred to 70 per cent alcohol until ready for use. In this way many of the organic substances, which would have been precipitated by the alcohol, were dissolved out. The alcohol whitened the nerves and made them stand out in contrast with the other tissues. It was difficult to determine the proper length of time to continue the maceration, as it varied with different specimens and with the temperature. A number were tried and the best selected.

Dissection was carried on under water or weak alcohol with the aid of a lens, fine pointed forceps, and needles. Whenever any doubt arose in regard to the character of the tissues, the doubtful portions were excised, stained, and examined under a compound microscope.

Injected specimens were used in tracing out the arteries. A great deal of the work of dissection was verified by examination of Dr. Patten's serial sections, both longitudinal and transverse, of young crabs, from 1 to  $1\frac{1}{2}$  inches long exclusive

of the caudal spine. These crabs had been imbedded in celloidin, sectioned, and stained in borax carmine, or in haematoxylin and picro-acid-fuchsin.

The histology of the heart, pericardium, alary muscles, etc., was studied by means of sections and pieces, excised, stained, and mounted. The heart with the neighboring tissues was cut out and hardened in Flemming's strong solution. Heidenhain's iron haematoxylin and eosin gave in most cases the best stain for sections; Kleinenberg's haematoxylin and eosin were, also, found very satisfactory.

The nerves upon the heart were made out by means of Löwit's gold chloride method, and the results verified by the methylen blue method.

*Gold Chloride Method.*—The hearts of young Limuli, about 5 inches in length, were dissected out, slit open along the ventral side, spread out, and treated with formic acid (one volume of water to one of the acid) until the tissues became transparent. They were then put for fifteen minutes or half an hour in 1 per cent gold chloride solution, and afterwards left in the dark for twenty-four hours in dilute formic acid (one part acid to three of water). Finally, they were put into strong formic acid, and left for three or four days in the dark, until the muscles on the inside of the heart had macerated to such an extent that they could be washed away by careful manipulations of the pipette, leaving the nerve plexus intact. The hearts were then spread out on slides and mounted in glycerine, acidulated with formic acid.

*Methylen Blue Method.*—Methylen blue was used in various ways. Some very good stains of the nerves in the appendages and upon the heart were obtained by injecting small crabs with a 1 per cent aqueous solution.

The injection method proved successful in only a small percentage of cases. More uniform results were obtained by immersing portions of the crabs in a solution of methylen blue in serum, a method first used by Dr. Patten. The serum was obtained from the clotted blood of large Limuli. Enough of the stain was dissolved in the serum to give it a clear blue color, and the solution was kept well oxygenated by forcing air into



it with a pipette. The tissues to be stained were kept exposed to the air and moistened from time to time with the solution. This method almost invariably gave a stain of the nerves in from ten minutes to one hour.

The nerves upon the heart, pericardium, oesophagus, pro-ventriculus, rectum, gills and sides of the carapace (after stripping off the chitin) were easily stained by this method.

As yet all attempts to obtain a stain of the nerves upon the intestine have failed.

## I. PRELIMINARY DESCRIPTION OF THE ANATOMY OF LIMULUS.

### I. EXTERNAL FORM.<sup>1</sup>

The carapace of *Limulus* has been divided by Lankester into three regions: (1) the prosomatic carapace or cephalothorax (Pls. VI and VIII, Figs. 1 and 3, *pros.*) ; (2) the meso-metасomatic or abdominal carapace (Pls. VI and VIII, Figs. 1 and 3, *mes.*) ; and (3) the postanal spine, caudal spine or telson (Pls. VI-IX, Figs. 1-5, *tel.*). The terms "haemal" and "neural" will be substituted for dorsal and ventral, in the following descriptions.

The cephalothorax bears upon its haemal surface two large, lateral, compound eyes (Pls. VI and VII, Figs. 1 and 2, *l.e.*) and three simple, median eyes (Pl. VIII, Fig. 3, *m.e.*). Early observers found only two ocelli, but Patten (*Quar. Jour. Micr. Sci.*, 1893) described two ectoparietal eyes, and an endoparietal eye formed of two retinas fused together.

The neural surface of the cephalothorax bears the olfactory organs, mouth, nephridial openings, and seven pairs of appendages ; *viz.*, the chelicerae, five pairs of ambulatory legs, and the chilaria.

The olfactory organs (Pls. VII and VIII, Figs. 2 and 3, *ol.or.*) lie in the median line anterior to the chelicerae, and about one-third the distance between the bases of the chelicerae

<sup>1</sup> For a more detailed description, see Lankester, "*Limulus* an Arachnid," *Quar. Jour. Micr. Sci.*, and Benham, "Muscular and Endoskeletal Systems of *Limulus*," *Trans. Zool. Soc.*, London, 1885.

and the anterior edge of the carapace (see Patten, *Quar. Journ. Micr. Sci.*, 1893). The seven pairs of appendages surround the mouth (Pl. VIII, Fig. 3, *m.*), which lies nearly in the center of the cephalothorax. The nephridial openings (Pl. VII, Fig. 2, *n.o.*) may be seen just back of the fifth pair of legs.

The abdomen (Pls. VI and VIII, Figs. 1 and 3, *mes.*) is attached to the cephalothorax by a transverse hinged joint on the haemal side of the animal, and is capable of movement in a haemo-neural direction only. It bears on its lateral edges six pairs of spines (Pl. VI, Fig. 1, *a.s.*<sup>9-14</sup>), the first pair belonging to the first branchial metamere, and the last pair to the first post-branchial metamere.

On the neural surface of the abdomen are six pairs of lamellar appendages (Pl. VIII, Fig. 3, *ap.*<sup>8-13</sup>); the genital openings are on the posterior side of the first pair of abdominal appendages, and the anus (Pls. VI and VIII, Figs. 1 and 3, *a.*) is at the base of the caudal spine.

The caudal spine (Pls. VI, VIII, and IX, Figs. 1, 3-5, *tel.*), or telson, is a long, sword-shaped, terminal segment capable of movement upon the abdomen in any direction.

*Entapophyses*.—Seven pairs of entapophyses, or chitinous infoldings of the haemal side of the carapace (Pls. VI, VIII, and IX, Figs. 1, 4-6, *enta.*<sup>7-14</sup>), serve for the attachment of muscles. These infoldings may be seen from the exterior; one pair (*enta.*<sup>7-8</sup>) on the cephalothorax, just anterior to the hinge, and the other six pairs (*enta.*<sup>9-14</sup>) upon the abdomen. The first pair (*enta.*<sup>7-8</sup>), which are much larger than the others, are probably formed by the fusion of the two pairs belonging to the chilial and opercular metameres; the next five pairs (*enta.*<sup>9-13</sup>) upon the abdomen belong to the five gill metameres; the last pair (*enta.*<sup>14</sup>) belong to the first post-branchial metamere.

*Tendinous Stigmata*.—Six pairs of chitinous infoldings of the neural surface of the abdomen, close behind the bases of the six pairs of abdominal appendages, serve for the attachment of the branchio-thoracic muscles. They have received the name of *tendinous stigmata* (Pls. VI and IX, Figs. 1 and 6, *t.s.*<sup>8-13</sup>).

*The Appendages*.—In describing the appendages it seemed desirable to consider them all as turned outward so as to lie at



right angles to the median line and parallel to each other. The homologies can then be more readily made out. The first pair, the chelicerae (Text-fig. 1; Pl. VIII, Fig. 3, *ap.*<sup>1</sup>), lies almost parallel to the median line, so that the morphologically anterior side faces the median line. They are small chelate appendages, anterior to the mouth, consisting of but three segments, and are regarded as homologous with the antennae of insects and myriapods.

The next four pairs of appendages (Text-fig. 2), from the second to the fifth, serve as ambulatory legs and also as masticatory organs. In the female they are all chelate. In the male the chelae of the second pair of appendages are modified to serve as clasping organs. The propodite is thickened, and its terminal process, which is ordinarily the anterior blade of the chela, is aborted; the dactylopodite, or posterior blade of the chela, is curved anteriorly so as to be opposed to the aborted end of the propodite.

Each of these appendages is composed of six joints, of which the fourth is double, being formed by the fusion of the meropodite and the carpopodite. The proximal margin of the coxopodite (Text-fig. 2, *I-cox.*) is much thickened, and forms a structure of complicated outline which has been called by Lankester the "entocoxite." If the coxopodite be examined from the proximal side, the entocoxite may be clearly seen (Pls. VI and VII, Figs. 1 and 2, *ent.*<sup>2-6</sup>). The outer portion is divided by chitinous bars into three spaces filled with areolar tissue containing numerous nerve endings. These spaces appear as slight swellings or knobs, probably highly sensory, upon the outer extremity of the base of the coxopodite. The inner, or median, portion of the coxopodite is modified to form a mandible (Text-fig. 2, *man.*) which projects over the mouth and bears numerous, inwardly projecting spines provided with gustatory buds. The mandibles of the third, fourth, and fifth pairs of appendages bear an inner detached portion (Text-fig. 2, *i.man.*) furnished with a small flexor muscle. These inner mandibles have been called by Lankester the "epicoxites." They are also supplied with spines and gustatory buds.<sup>1</sup>

<sup>1</sup> For the structure of the gustatory buds of the mandibles, and also of similar buds in the chelae, see Patten, "Morphology and Physiology of the Brain and Sense Organs of *Limulus*," *Quar. Journ. Micr. Sci.*, 1893.

A chitinous infolding, or apodeme (Text-fig. 2, *apo.*), arises from the arthroidal membrane, between the third and fourth joints, and projects into the cavity of the third joint. It furnishes attachment for a large flexor muscle arising from the anterior side of the second joint.

The sixth pair of appendages (Text-fig. 3) are the powerful legs used for burrowing and pushing the animal along through the sand. The inner mandibular portion (Text-fig. 3, *man.*) lacks gustatory spines, is very massive, and serves as a crushing jaw. Two of the sensory knobs of the outer portion of the coxopodite are like the corresponding parts of the other ambulatory legs, but the third or median one is replaced by a spatulate organ, the flabellum (Text-fig. 3, *flab.*, and Pls. VI and VII, Figs. 1 and 2, *ent.*<sup>6</sup>).

The homologies of these sensory knobs will be discussed later, under the nervous system.

The fifth joint (Text-fig. 3, *5-pro.*), instead of forming with the sixth a chela, as in the other legs, is oblong in longitudinal section, and bears upon its distal end a rosette of four shorter spatulate organs, and the sixth joint. The latter (Text-fig. 3, *6-dac.*) bears at its distal end two small terminal joints, which are opposed to each other and function as a small chela.

The chilaria (Text-fig. 4), which lie posterior to the mouth, are a pair of small appendages consisting of a single segment. Owen regarded them as detached portions of the sixth pair of appendages, but they undoubtedly represent true appendages and belong to a distinct metamere.

The first pair of appendages upon the abdomen (Text-fig. 5) are almost completely fused in the median line, and form a large operculum, which overlaps the five pairs of gills. Each half consists of a large proximal portion marked off by radiating creases into triangular areas, and a small distal portion divided into two lobes—an inner lobe, or endopodite (Text-fig. 5, *i.l.*), consisting of two joints, and an outer lobe, or exopodite (*o.l.*), consisting of one joint. Two genital papillae upon the posterior side of the proximal portion mark the external openings of the oviducts (Text-fig. 5, *ov.*). There is no gill book upon this appendage.











The remaining five pairs of abdominal appendages (Text-fig. 6) are alike in form, but decrease in size towards the posterior end of the body. A deep median cleft divides them into right and left halves, between which in the median line is inserted a membranous tongue or median lobe (Text-fig. 6, *m.l.*). The basal portion of each half bears a gill book (Text-fig. 17, *g.b.*) consisting of numerous overlapping leaflets. The endopodite (*i.l.*) is slender, and produced a short distance beyond the broad exopodite or outer lobe (*o.l.*).

## 2. THE ENDOSKELETAL SYSTEM.

In *Limulus* there are a number of cartilaginous bodies which serve for the attachment of muscles. These are the plastron, or endocranium (Text-figs. 2-5; Pls. VI and VIII, Figs. 1, 3, and 4, *endo.*); six small abdominal endochondrites (Text-figs. 5 and 6; Pls. VI, VIII, and IX, Figs. 1, 3, 4, and 6, *a.e.<sup>8-13</sup>*), one at the base of each pair of abdominal appendages; and six pairs of branchial bars (Text-figs. 5 and 6; Pls. VI and IX, Figs. 1 and 6, *b.c.<sup>8-13</sup>*) supporting the operculum and gills. Another pair of branchial bars (*b.c.<sup>7</sup>*) supporting the chilaria are fused with the endocranium.

### a. *The Endocranium.*

The endocranium has been fully described in the first paper of this series. Here we shall merely state that this piece of cartilage serves as a centrum for the attachment of the longitudinal abdominal muscles (Text-figs. 5 and 6; Pls. VI, VIII, and IX, Figs. 1, 4, and 6, *l.a.m.*), tergo-plastrals (Pls. VIII and IX, Figs. 4 and 5, *d.l.p.t.*), tergo-proplastrals (Text-fig. 8; Pls. VI, VIII, and IX, Figs. 1, 4, and 5, *t.p.m.<sup>a-c</sup>*), veno-pericardiac muscles (Text-fig. 4; Pl. VIII, Fig. 4, *v.p.m.<sup>6-7</sup>*), and numerous muscles inserted upon the coxopodites of the appendages, from second to the seventh, inclusive.

A pair of bars (Text-figs. 4 and 5; Pls. VI, VII, and VIII, Figs. 1-4, *b.c.<sup>7</sup>*) of capsuliginous cartilage, identical with that of the branchial bars of the abdominal appendages, are fused,

proximally, with the posterior border of the endocranium and are attached distally to the posterior sides of the chilaria.

Near the posterior extremity of the endocranium are two pairs of foramina (Text-figs. 3-5; Pls. VI and VIII, Figs. 1, 3, and 4, *f.*<sup>6-7</sup>) which afford a passage for intestinal nerves (*i.n.*<sup>9-7</sup>).

#### b. *The Abdominal Endochondrites.*

These are six in number (Text-figs. 5 and 6; Pls. VI, VIII, and IX, Figs. 1, 3, 4, and 6, *a.e.*<sup>8-13</sup>), metamerically arranged in the median line neural to the ventral cord, one at the base of each pair of abdominal appendages (*ap.*<sup>8-13</sup>). It will be noticed that they lie upon the side of the central nervous system opposite to that on which the endocranium lies. They are fibroid in structure, like the endocranium, and act as centra for the attachment of the internal branchial muscles (Text-figs. 5 and 6; Pl. IX, Fig. 6, *i.b.m.*<sup>8-13</sup>), longitudinal abdominal muscles (Text-figs. 5 and 6; Pls. VI, VIII, and IX, Figs. 1, 4, and 6, *l.a.m.*), and haemo-neural muscles (Pls. VI, VIII, and IX, Figs. 1, 4-6, *h.n.m.*<sup>8-13</sup>).

#### c. *The Branchial Cartilages.*

These structures were described by Gegenbaur in 1858. Lankester (*Quar. Journ. Micr. Sci.*, 1884) described a "pair of ligamentous bands, the *entapophysial ligaments*, which pass from one to another of the dorsal ingrowths of the integument known as the dorsal entapophyses." "The ligamentous band is not of equal dimensions throughout, but where it is attached to an entapophysis it gives off at right angles a conical, knob-like protuberance."

This ligament is continuous only between the first and second pairs of entapophyses. From the outer side of every entapophysis except the seventh, a cartilaginous bar (Text-figs. 5 and 6; Pls. VI and IX, Figs. 1 and 6, *b.c.*<sup>8-13</sup>) passes neurally to the inside of the appendage of the same metamere *ap.*<sup>8-13</sup>. It acts as a skeletal support for the appendage and also for the attachment of numerous muscles. These bars consist of a highly characteristic core of capsuliginous cartilage enveloped

in a tough fibroid cortical layer. The same kind of cartilage is found in the structures arising from the posterior portion of the endocranium and attached to the insides of the chilaria.

### 3. THE MUSCULAR SYSTEM.

Lankester (*Trans. Zool. Soc.*, London, 1885), with the assistance of Mr. Benham, gave a detailed account of the muscular system of *Limulus*. He, however, omitted the chilarial and the anal muscles, as well as the muscles of the thoracic appendages, except the coxal muscles. The anal muscles were figured by A. Milne-Edwards (*Ann. Sci. Nat.*, 1873).

In the cephalothorax the edges and neural surface of the endocranium afford attachment for a great many muscles which radiate to the bases of the ambulatory legs and are inserted upon the inner proximal portions of the coxopodites from the second to the sixth, inclusive. Lankester calls them the *plastro-coxal* muscles (Text-figs. 2 and 3; Pl. VII, Fig. 2, *3<sup>a</sup>* and *b*, *e* and *g*, *6<sup>a</sup>*, *b*, *e*, *f*, and *g*, and corresponding muscles in the second to the sixth appendages). They assist in the complicated chewing movements of the mandibles.

Three pairs of muscles, the *tergo-proplastrals* (Text-fig. 13; Pls. VI, VIII, and IX, Figs. 1, 4, and 5, *t.p.m.<sup>a-c</sup>*), suspend the anterior cornua of the plastron from the haemal portion of the carapace.

A small muscle, *lateral proplastro-tergal*, fastens each of the lateral cornua to the haemal side of the carapace.

A pair of *plastro-tergals* (Pls. VIII and IX, Figs. 4 and 5, *d.l.p.t.*) attach the haemal processes of the endocranium to the carapace, and a pair of *plastro-entapophysials* (Pl. VIII, Fig. 4, *d.l.p.e.*) pass from the same processes to the first pair of entapophyses.

From the posterior portion of the haemal surface of the endocranium large *meso-plastro-entapophysials* (Pl. VIII, Fig. 4, *m.p.e.*) also go to the first pair of entapophyses. All of these muscles serve to suspend the endocranium in a firm position in the cephalothorax and to counteract the contractions of the coxal muscles and longitudinal abdominal muscles, which would

tend to draw the endocranium out of place. They may also serve to compress the cephalothorax and thus aid in the expulsion of the genital products.

A few muscle strands, *plastro-buccal muscles*, go from the anterior neural side of the plastron to the oesophagus, and a few more, beneath the skin behind the mouth, go from the occipital ring to the oesophagus.

A mass of *longitudinal abdominal muscles* (Text-figs. 5 and 6; Pls. VI and VIII, Figs. 1 and 4, *l.a.m.*) arises from the posterior haemal side of the endocranium and passes backward, giving off slips to each pair of abdominal entapophyses and to the abdominal endochondrites. It terminates upon the integument just posterior to the last pair of gills. Portions of this mass join together the successive endochondrites. A pair of slips are inserted upon the integument posterior to each of the four gills and just median to the infoldings of the tendinous stigmata. They unite with the terminal portion of the mass of abdominal muscles, pass backward, and are attached to the integument posterior to the last pair of gills.

Four *inter-entapophysial* muscles (Pl. VIII, Fig. 4, *i.e.m.*) pass from the first pair of entapophyses to the next four pairs.

The tendinous stigmata (*t.s.*<sup>8-13</sup>) of the six pairs of abdominal appendages furnish attachment for a bundle of *branchio-thoracic* muscles (Text-figs. 5 and 6; Pls. VI and IX, Figs. 1, 5, and 6, *b.t.m.*) which run forward just neural and median to the row of entapophyses, and external to the large bundle of abdominal muscles proceeding from the endocranium. After passing median to the tergo-coxal muscles, external to the tergo-plastrals, and haemal to the plastro-coxals, the branchio-thoracic muscles attach themselves by two slips (Pl. IX, Fig. 5, *b.t.m.*<sup>a</sup> and <sup>b</sup>) to the haemal side of the carapace, external to the pericardial sinus (*p.s.*).

Between the branchio-thoracic muscles and the longitudinal abdominal muscles is a double membrane closely investing these muscles like a perimysium, and affording attachment for the veno-pericardiac muscles (see Pl. IX, Fig. 6, *v.p.m.*<sup>9</sup>).

All of these longitudinal muscles act together as flexors of the abdomen.



The abdomen is extended by two pairs of powerful *inter-tergal* muscles, Pls. VIII and IX, Figs. 4 and 5, *i.m.* (only the external ones are represented) in the median line on the haemal side of the animal. The external pair of muscles arise from the median faces of the first pair of entapophyses, and the internal pair from an extended area upon the haemal, median portion of the carapace. Both pairs are inserted in the median line on the anterior, haemal border of the abdominal carapace.

Seven pairs of *haemo-neural muscles* (Pls. VI, VIII, and IX, Figs. 1, 4-6, *h.n.m.*<sup>8-14</sup>) arise from the haemal side of the abdominal carapace. The first six pairs (*h.n.m.*<sup>8-13</sup>) are inserted upon the six abdominal endochondrites, and hence belong to the opercular and five branchial metameres, respectively. The seventh pair (*h.n.m.*<sup>14</sup>) are inserted upon the neural side of the carapace posterior to the last appendage. The first pair (*h.n.m.*<sup>8</sup>), which belong to the opercular metamere, arise upon the anterior border of the abdominal carapace, from the median side of a pair of small protuberances which may possibly represent the remnants of a pair of entapophyses, though it is more likely that the entapophyses of the chilial and opercular metameres have fused with each other. As will be seen later, the fact that there is a fusion of other structures in this region serves to support this hypothesis. The remaining six pairs of haemo-neural muscles (*h.n.m.*<sup>9-14</sup>) arise from the carapace on the median side of the six pairs of abdominal entapophyses.

The haemo-neural muscles aid in holding the endochondrites in place by counteracting the contractions of the internal branchial muscles (*i.b.m.*) which arise from the endochondrites. They also serve to compress the abdomen.

Eight pairs of *veno-pericardiac* muscles (Text-fig. 4; Pls. VIII and IX, Figs. 4 and 6, *v.p.m.*<sup>6-13</sup>), "brides transparentes," of Milne-Edwards, are attached to the neural side of the pericardium opposite the eight pairs of ostia (*os.*<sup>6-13</sup>) of the heart. These muscles pass neurally on each side of the intestine to the integument upon the neural side of the body. Instead of being attached directly to the integument, however, the bases of the muscles expand and become continuous with a tough connective-tissue membrane running longitudinally between the branchio-

thoracic muscles and the longitudinal abdominal muscles. In the abdominal region this membrane is double, the two portions partially investing the branchio-thoracic muscles on the one hand, and the longitudinal abdominal muscles upon the other. Between the successive appendages it is attached to the integument. The space between the two portions is the ventral collecting venous sinus (Pl. IX, Fig. 6, *v.c.s.*), which, with its fellow upon the opposite side of the body, carries the blood to the gills to be aerated. In the thoracic region the membrane does not enclose a venous sinus. It is single and attached to the sides of the endocranium, and, in places, to the integument between the plastro-coxal muscles.

The caudal spine is moved by various muscles which arise by a large number of slips, each of which Lankester has designated by a special name. For our purpose, however, it will be better to divide them into three groups and consider each group as one pair of muscles, thus making one pair of flexors and two pairs of extensors. The pair of flexors (Pl. VI, Fig. 1, *t.f.m.*) arise by numerous slips from the neural portion of the abdominal carapace posterior to the appendages, and from the outer and posterior sides of the last three pairs of entapophyses, and are inserted upon the outer neural portions of the arthroidal membrane which attaches the caudal spine to the abdomen.

One pair of extensors (Pl. IX, Fig. 5, *t.e.m.<sup>a</sup>*) arise by numerous slips from the haemal side of the abdomen posterior to the heart, and from the inner sides of the last three pairs of entapophyses, and are inserted upon the haemal portion of the above-mentioned arthroidal membrane, near the median line. The second pair of extensors (Pls. VI and IX, Figs. 1 and 5, *t.e.m.<sup>b</sup>*) arise upon both the haemal and neural sides of the carapace, and are inserted upon the arthroidal membrane, external to the first pair of extensors.

Coördinate action of the muscles on either side of the median line moves the caudal spine in a lateral direction.

A *sphincter* muscle (Pls. VI and VIII, Figs. 1, 3 and 4, *s.a.*) closes the anus. A pair of slender muscles (Pl. VI, Fig. 1, *o.a.*), which may be called the *occludor ani*, arising on the neural side of the carapace, just posterior to the last appendage, are inserted











upon the anterior side of the proctodaeum, close to the anus. These serve to draw the anus forward, and by elongating the anal slit possibly assist the *sphincter ani* in its function. A pair of band-shaped muscles (Pls. VI, VIII, and IX, Figs. 1, 3-5, *l.a.*), or *levator ani*, are inserted upon the sides of the anus, and pass haemally and outward to the haemal side of the carapace. They serve to open the anus.

*The Cheliceral Muscles* (Text-fig. 1).—In the chelicerae the first joint is moved by four muscles (Text-fig. 1, *e.<sup>1</sup>*, *f.<sup>1</sup>*, and *l.m.<sup>1</sup>*). Lankester regarded these as one muscle and called it the tergo-coxal muscle of the chelicera. They have their origin on the haemal side of the carapace, descend vertically on the median side of the anterior cornua of the endocranium, and are inserted upon the base of the first joint of the appendage. The flexor (*f.<sup>1</sup>*) is inserted upon the inner margin, the extensor (*e.<sup>1</sup>*) upon the outer margin, and a small muscle (*l.m.<sup>1</sup>*) upon the sides midway between the flexor and extensor muscles. In these descriptions the appendages are supposed to be revolved outward, so as to lie at right angles to the median line and parallel to the other appendages. The flexor and extensor muscles act in opposition to each other and give to the whole appendage a movement toward or away from the mouth. The small lateral muscles give it a slight lateral movement.

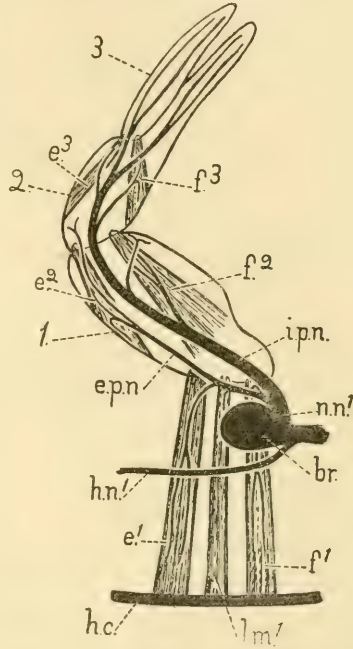


FIG. 1.—Diagram showing muscles and distribution of nerves in chelicera of adult *Limulus*, from the anterior side (natural size). 1, 2, and 3, first, second, and third joints of chelicera; *e.<sup>1-3</sup>*, extensors of first, second, and third joints, respectively; *f.<sup>1-3</sup>*, flexors of first, second, and third joints, respectively; *l.m.<sup>1</sup>*, lateral muscles of first joint; *e.<sup>1</sup>*, *f.<sup>1</sup>*, and *l.m.<sup>1</sup>* constitute the tergo-coxal muscles; *h.c.*, haemal side of the carapace; *br.*, fore-brain; *n.n.*, neural nerve or cheliceral nerve; *i.p.n.*, internal pedal nerve; *e.p.n.*, external pedal nerve; *h.n.*, haemal nerve of cheliceral neuromere or lateral nerve.

The second joint is moved by two muscles. A large flexor (*f.*<sup>2</sup>) arises from the anterior side of the proximal joint, and is inserted upon the inner proximal margin of the second. A smaller extensor muscle (*e.*<sup>2</sup>) arises from the outer and posterior sides of the proximal joint, and is inserted upon the outer

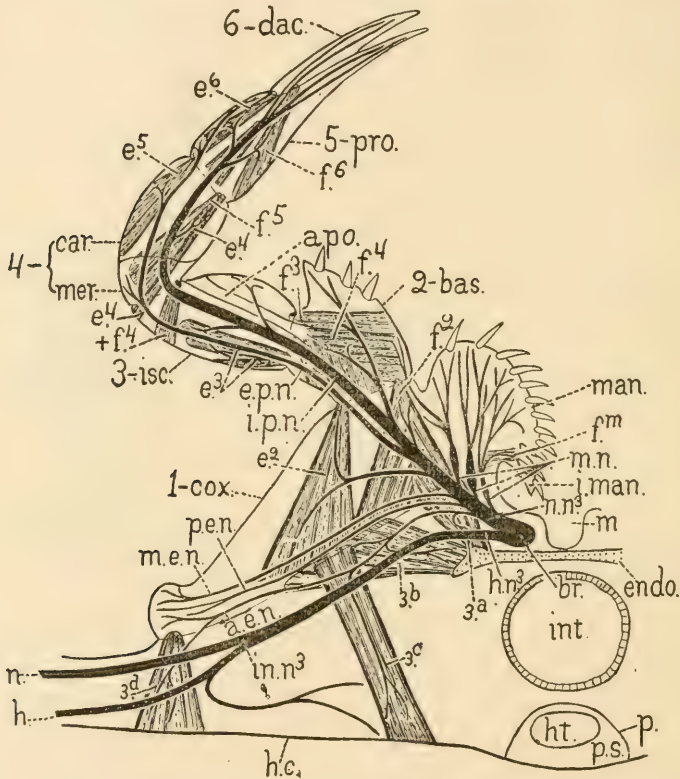


FIG. 2.— Diagram showing muscles and distribution of nerves in the third leg of *Limulus*, from the anterior side ( $\frac{3}{4}$  natural size).

1-cox., coxopodite, or first joint; 2-bas., basipodite, or second joint; 3-isc., ischopodite, or third joint; 4-mer., fused carpopodite and meropodite, or fourth joint; 5-pro., propodite, or fifth joint; 6-dac., dactylopodite, or sixth joint; apo., apodeme; h.c., haemal side of carapace; endo., endocranium; ht., heart; int., intestine; i.man., internal mandible; m., mouth; man., mandible; p., pericardium; p.s., pericardial sinus.

MUSCLES: 3<sup>a</sup> and b, plastro-coxal muscles inserted upon anterior side of entocoxite; 3<sup>c</sup> and d, tergo-coxal muscles inserted upon anterior side of entocoxite; e.<sup>2-6</sup>, extensors of second to sixth joints; f.<sup>2-6</sup>, flexors of second to sixth joints; f.m., flexor of inner mandible.

NERVES: a.e.n., anterior ento-coxal nerve; br., brain; e.p.n., external pedal nerve; h., haemal branch of integumentary nerve; h.n.<sup>3</sup>, haemal nerve; i.n.<sup>3</sup>, integumentary branch; i.p.n., internal pedal nerve; m.e.n., median ento-coxal nerve; m.n., mandibular nerves; n., neural branch of integumentary nerve; n.n.<sup>3</sup>, neural nerve; p.e.n., posterior ento-coxal nerve.



proximal margin of the second. These muscles move the chela toward and away from the mouth.

The third joint, or movable blade of the chela, is moved by two muscles, — a large flexor ( $f.^3$ ) arising from the anterior and inner sides of the second joint and inserted upon the anterior proximal margin of the third, and a small extensor ( $e.^3$ ) arising from the posterior side of the second joint and inserted upon the posterior proximal margin of the third. By these muscles the third joint is moved at right angles to the movements of the other joints of the appendages, and the chela opened and closed.

*The Muscles of the Second, Third, Fourth, and Fifth Appendages* (Text-fig. 2). — As these appendages are very similar in their musculature, the third one may be taken as a type, and the others compared with it. The muscles which move the coxopodite are nine in number. Four plastro-coxals (Text-fig. 2,  $3^{a \text{ and } b}$ ; Pl. VII, Fig. 2,  $4^{a, b, c, \text{ and } d}$ ) arise from the side of the plastron and are inserted upon the median half of the entocoxite, two on the anterior side and two on the posterior. The remaining five (Text-fig. 2,  $3^{c \text{ and } d}$ ; Pl. VII, Fig. 2,  $4^{c, d, h, \text{ and } j}$ ) arise from the haemal side of the carapace and are inserted upon the outer portion of the entocoxite. Two of these,  $4^c$  and  $4^d$ , or  $3^c$  and  $3^d$ , are inserted upon the anterior side, and three,  $4^h$ ,  $4^i$ , and  $4^j$ , upon the posterior.

In the second appendage the muscle corresponding to  $4^c$  is absent or fused with the one corresponding to  $4^d$ . In the sixth appendage there is an extra plastro-coxal muscle,  $6^f$ , on the posterior side, arising from a point on the plastron much farther forward than the origins of the other plastro-coxals of that appendage. The tergo-plastral muscle corresponding to  $4^h$  is apparently fused with  $6^i$ . All of these muscles assist in performing the complicated chewing movements of the coxopodites as well as the forward and backward movements of the legs in walking and swimming.

The second joint, or basipodite (Text-fig. 2, *2-bas.*), is moved by two muscles: a large flexor ( $f.^2$ ) arising from the posterior side of the coxopodite (*1-cox.*) and inserted on the inner proximal margin of the basipodite; a smaller extensor ( $e.^2$ ) arising

from the anterior side of the coxopodite and inserted upon the outer proximal margin of the basipodite.

The third joint, or ischiopodite (*3-isc.*), is moved by a more complicated set of muscles. A large flexor muscle (*f.<sup>3</sup>*) arises from the posterior side of the basipodite, and is inserted upon the inner proximal margin of the ischiopodite. Another large muscle (*f.<sup>4</sup>*), arising from the anterior side of the basipodite, is inserted upon a chitinous infolding of the arthroidal membrane (*apo.*) on the median side of the appendage between the ischiopodite and the mero-carpopodite. This muscle acts as a flexor of the mero-carpopodite, and also as an extensor of the ischiopodite. Three small muscles (*e.<sup>3</sup>*), arising from the outer side of the ischiopodite, and inserted upon the outer margin of the distal portion of the basipodite, act as extensors of the ischiopodite.

Besides the large flexor of the mero-carpopodite, above mentioned, a pair of small flexors (*f.<sup>4</sup>*) of the same joint arise from the outer side of the ischiopodite, and are inserted upon the inner margin of the proximal portion of the mero-carpopodite. A pair of slender muscles (*e.<sup>4</sup>*) arising from the outer side of the ischiopodite, near the distal end, and inserted upon the inner margin of the proximal portion of the propodite, act as extensors of the mero-carpopodite, and also as flexors of the propodite.

Another pair of flexors (*f.<sup>5</sup>*) of the propodite arise from the outer side of the mero-carpopodite, and are inserted upon the inner proximal margin of the propodite. The extensors (*e.<sup>5</sup>*) of the propodite are a pair of muscles arising from the outer side of the mero-carpopodite, and inserted upon the outer proximal margin of the propodite. The propodite is capable of movement upon the mero-carpopodite in any direction. Movement in an anterior direction is effected by the coördinate contraction of the anterior extensors and flexors, and movement in a posterior direction, by contraction of the posterior extensors and flexors.

The dactylopodite (*6-dac.*) is moved by a large flexor (*f.<sup>6</sup>*) arising from the anterior, inner, and outer sides of the propodite, and inserted on the anterior proximal margin of the dactylopo-

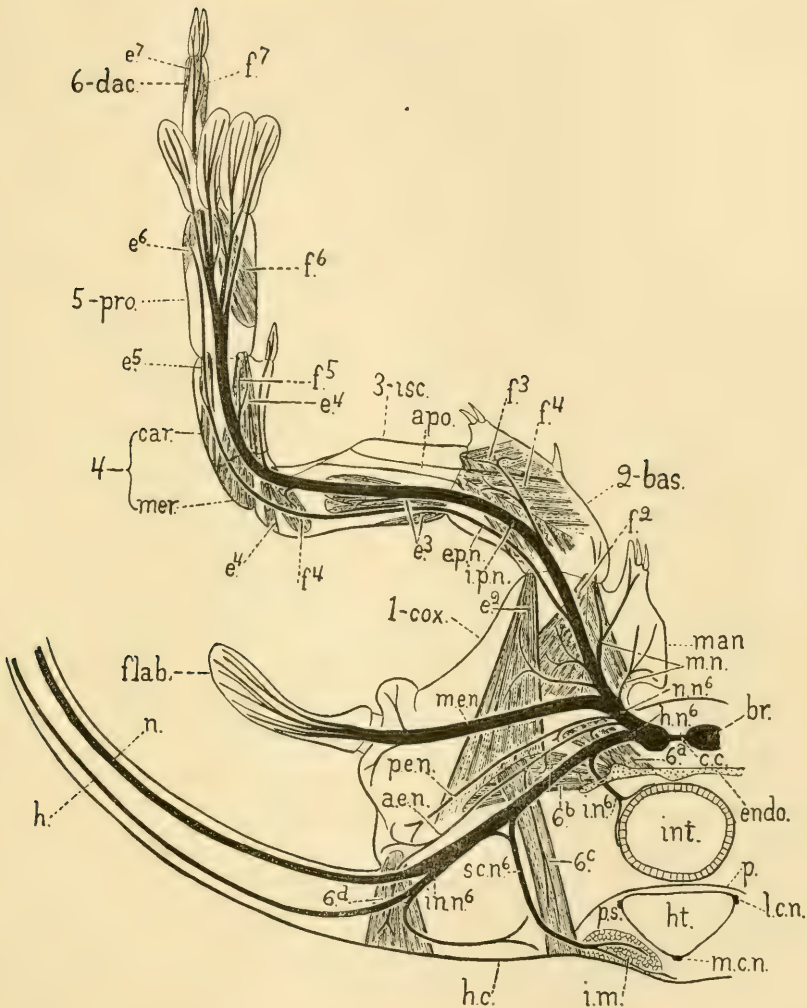


FIG. 3.—Diagram showing the muscles and distribution of the nerves in the sixth leg of *Limulus*, from the anterior side ( $\frac{2}{3}$  natural size).

1-cox., coxopodite, or first joint; 2-bas., basipodite, or second joint; 3-isc., ischiopodite, or third joint; 4- { car., fused carpopodite and meropodite, or fourth joint; 5-pro., propodite, or fifth joint; 6-dac., dactylopodite, or sixth joint; apo., apodeme; br., brain; c.c., cross-commis-  
sure; endo., endocranium; flab., flabellum; h.c., haemal side of carapace; ht., heart; int., intestine; man., mandible; p.s., pericardial sinus.

MUSCLES: 6a and b, plastro-coxal muscles inserted upon anterior side of entocoxite; 6c and d, tergo-coxal muscles inserted upon anterior side of entocoxite; e<sup>2-7</sup>, extensors of second to seventh joints; f<sup>2-7</sup>, flexors of second to seventh joints; i.m., inter-tergal muscle.

NERVES: a.e.n., anterior ento-coxal nerve; e.p.n., external pedal nerve; h., haemal branch of integumentary nerve; h.n.<sup>3</sup>, haemal nerve; i.n.<sup>6</sup>, intestinal nerve; i.n.<sup>6</sup>, integumentary branch of haemal nerve; i.p.n., internal pedal nerve; l.c.n., lateral cardiac nerve; m.c.n., median cardiac nerve; m.e.n., median ento-coxal nerve or flabellar nerve; m.n., mandibular nerve; n., neural branch of integumentary nerve; n.n.<sup>6</sup>, neural nerve; p., pericardium; p.e.n., posterior ento-coxal nerve; s.c.n.<sup>6</sup>, segmental cardiac nerves.

dite, and by a small extensor ( $e.^6$ ) arising from the posterior side of the propodite and inserted upon the posterior proximal margin of the dactylopodite. These muscles close and open the chela.

A small flexor muscle ( $f.^m$ ) is found at the base of the inner mandible. This muscle is absent in the second appendage.

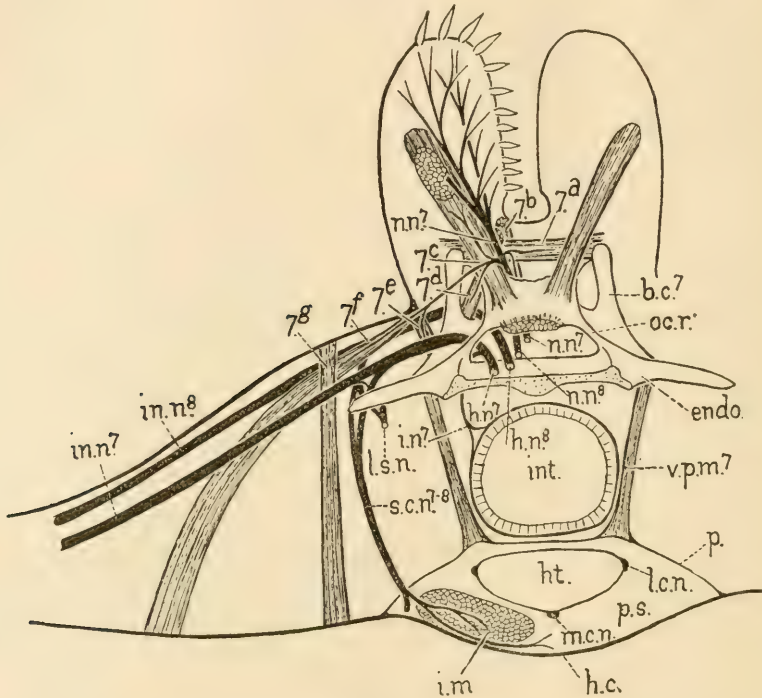


FIG. 4. — Diagram showing the muscles and nerves of the chilaria of *Limulus*, from anterior side. The appendages are revolved outward about  $45^\circ$  (magnified nearly  $1\frac{1}{2}$  diameters).

*b.c.7*, capsuliginous bar or branchial cartilage; *endo.*, endocranium; *h.c.*, haemal side of carapace; *ht.*, heart; *int.*, intestine; *oc.r.*, occipital ring; *p.s.*, pericardial sinus.

MUSCLES:  $7^a$ - $c$ , plastro-coxal muscles;  $7^f$  and  $g$ , tergo-coxal muscles; *i.m.*, inter-tergal muscles; *v.p.m.7*, veno-pericardiac muscles.

NERVES: *h.n.7* and  $8$ , haemal nerves of chilarial and opercular neuromeres; *i.n.7*, intestinal nerve; *in.n.7* and  $8$ , integumentary branches of haemal nerves of chilarial and opercular segments; *l.c.n.*, lateral cardiac nerve; *l.s.n.*, lateral sympathetic nerve; *m.c.n.*, median cardiac nerve; *n.n.7* and  $8$ , neural nerves of chilarial and opercular neuromeres; *p.*, pericardium; *s.c.n.7* and  $8$ , fused segmental cardiac nerves of chilarial and opercular neuromeres.

*The Muscles of the Sixth Appendage* (Text-fig. 3). — These are similar to the muscles of the second, third, fourth, and fifth appendages, except those which move the joints beyond the propodite. A large muscle ( $f.^6$ ), arising from the posterior,



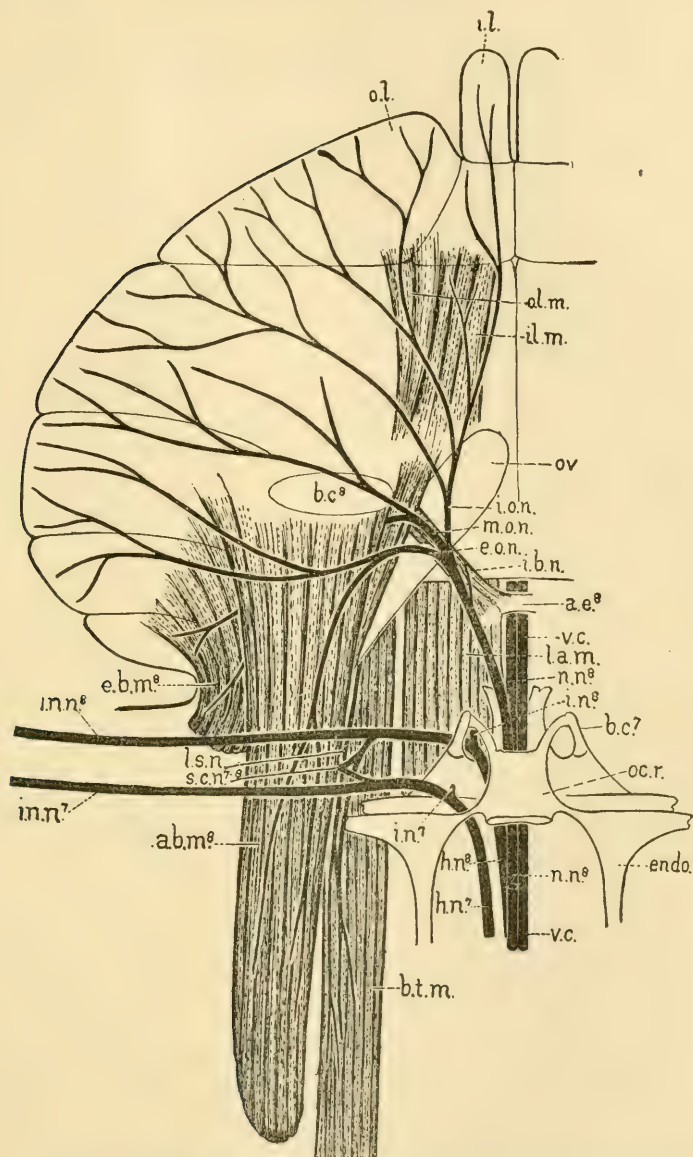


FIG. 5. — Diagram showing the muscles and distribution of the nerves in the operculum. The operculum is flexed upon the abdomen, and is seen from the neural side (about  $1\frac{1}{2}$  natural size).  
*a.e.*, abdominal endochondrite of opercular segment; *b.c.*<sup>7</sup>, capsuliginous bar or branchial cartilage of operculum; *endo.*, endocranium; *i.l.*, inner lobe of operculum; *oc.r.*, occipital ring; *o.l.*, outer lobe of operculum; *ov.*, oviduct.

MUSCLES: *a.b.m.*<sup>8</sup>, abductor muscle of operculum; *b.t.m.*, brachio-thoracic muscles; *e.b.m.*<sup>8</sup>, external branchial muscle; *i.b.m.*, internal branchial muscle; *i.l.m.*, muscle of inner lobe; *o.l.m.*, muscle of outer lobe.

NERVES: *e.o.n.*, external branch of opercular nerve; *h.n.*<sup>7</sup> and <sup>8</sup>, haemal nerves of chilarial and opercular neuromeres; *i.n.*<sup>7</sup> and <sup>8</sup>, intestinal nerves of chilarial and opercular neuromeres; *in.n.*<sup>7</sup> and <sup>8</sup>, integumentary branches of haemal nerves of chilarial and opercular neuromeres; *i.o.n.*, internal branch of opercular nerve; *l.s.n.*, lateral sympathetic nerve; *m.o.n.*, median branch of opercular nerve; *n.n.*<sup>8</sup>, neural or opercular nerve; *s.c.n.*<sup>7</sup> and <sup>8</sup>, fused segmental cardiac nerves of the seventh and eighth neuromeres; *v.c.*, ventral cord.

median, and anterior sides of the propodite, is inserted upon the arthroidal membrane between the bases of the whorl of spatulate organs upon the distal extremity of the propodite, and acts as a flexor for them all, as well as for the slender dactylopodite (*6-dac.*). A smaller muscle arising from the outer side of the propodite, and inserted upon the outer proximal margin of the dactylopodite, acts as the extensor of this joint, and also of the spatulate organs. The inner proximal margin of the dactylopodite extends like a spur toward the bases of the spatulate organs, and upon the contraction of the extensor muscle acts as a lever and lifts up the arthroidal membrane in the middle of the whorl of spatulate organs. This action tips them outward.

At the distal end of the longer joint are two smaller appendages opposed to each other like a chela, and opened and closed by small flexor (*f.*<sup>7</sup>) and extensor (*e.*<sup>7</sup>) muscles.

In the sixth appendage, as in the second, the inner mandible with its flexor muscle is absent.

*The Muscles of the Chilaria* (Text-fig. 4).—These muscles are comparable with those of the abdominal rather than with those of the thoracic appendages. A muscle (Text-fig. 4, Pls. VII and VIII, Figs. 2 and 3, *7*<sup>c</sup>) arises from the roof of the occipital ring, and is inserted by two slips to the posterior and anterior sides of the chilarium. This muscle draws the appendage forward. A few transverse strands of muscle fibers (*7*<sup>a</sup>) are attached to the posterior margins of the bases of the two chilaria, and draw the two appendages toward the median line. A muscle (*7*<sup>b</sup>) arises on the neural side of the posterior process of the endocranium, and is inserted upon the inner margin of the base of the chilarium.

Another small muscle (*7*<sup>c</sup>) arises on the inner side of the capsuliginous bar, and is inserted upon the base of the chilarium, close to the insertion of the last-described muscle. These two muscles aid in drawing the appendage backward and toward the median line. Still another small muscle (*7*<sup>e</sup>) arises from the posterior border of the endocranium, outside of the capsuliginous bar, and is inserted upon the outer margin of the base of the chilarium. A long muscle (*7*<sup>f</sup>) arises from the haemal side











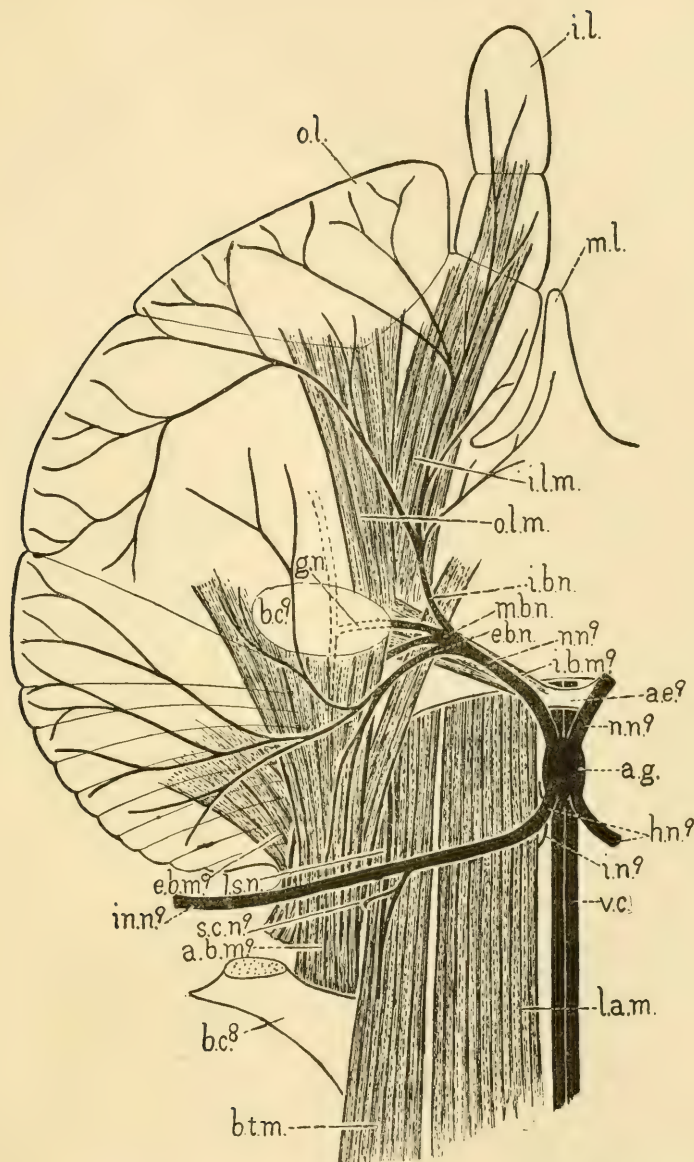


FIG. 6. — Diagram showing the muscles and distribution of nerves in the first gill. The appendage is flexed upon the abdomen, and is seen from the neural side (about  $1\frac{1}{2}$  natural size).

*a.e.*<sup>9</sup>, abdominal endochordite; *b.c.*<sup>8</sup> and <sup>9</sup>, branchial cartilages of operculum and first gill; *i.l.*, inner lobe of gill; *m.l.*, median lobe of gill; *o.l.*, outer lobe of gill.

MUSCLES: *a.b.m.*<sup>9</sup>, abductor muscle of gill; *b.t.m.*, branchio-thoracic muscles; *e.b.m.*<sup>9</sup>, external branchial muscle; *i.b.m.*<sup>9</sup>, internal branchial muscle; *i.l.m.*, inner lobe muscles; *l.a.m.*, longitudinal abdominal muscles; *o.l.m.*, outer lobe muscles.

NERVES: *a.g.*, first abdominal ganglion; *e.b.n.*, external branch of neural nerve; *g.n.*, branch of neural nerve supplying gill book; *h.n.*<sup>9</sup>, haemal nerves; *i.b.n.*, internal branch of neural nerve; *i.n.*<sup>9</sup>, intestinal nerve (two branches are shown, a posterior and an anterior one); *in.n.*<sup>9</sup>, integumentary branch of haemal nerve; *l.s.n.*, lateral sympathetic nerve; *m.b.n.*, median branch of neural nerve; *n.n.*<sup>9</sup>, neural nerve; *s.c.n.*<sup>9</sup>, segmental cardiac nerve; *v.c.*, ventral cord.

of the carapace, just anterior to the hinge, and passes haemally outside of the branchio-thoracic and longitudinal abdominal muscles to its insertion, close to that of the last-described muscle. These two muscles draw the appendage outward and backward.

Lastly, a muscle (7<sup>s</sup>) arising from the haemal side of the carapace passes neurally, and is inserted upon the integument a short distance from the base of the chilarium.

*The Muscles of the Operculum* (Text-fig. 5). — These have been minutely described by Lankester, so it will not be necessary to give them in detail. The appendages naturally lie flexed backward upon the abdomen. Large extensor, or abductor, muscles (*a.b.m.*<sup>8</sup>), arising from the haemal side of the cephalothorax and from the first entapophysis, are inserted upon the anterior lamella and upon the anterior side of the branchial bar (*b.c.*<sup>8</sup>) of the operculum. A large flexor, or adductor, muscle (Text-fig. 5; Pls. VI and IX, Figs. 1 and 5, *e.b.m.*<sup>8</sup>), arising from the haemal side of the abdominal carapace just posterior to the hinge, is inserted upon the posterior lamella and the posterior side of the branchial bar. A small internal branchial muscle (Text-fig. 5, *i.b.m.*) arises from the neural side of the first abdominal endochondrite (*a.e.*<sup>8</sup>) and is inserted upon the inner side of the branchial bar (*b.c.*<sup>8</sup>). The branchio-thoracic muscles have already been described. A few strands of muscles (*o.l.m.* and *i.l.m.*) flex and extend the inner and outer lobes of the appendage.

*The Muscles of the Gills* (Text-fig. 6). — These are similar to those of the operculum, except that the extensors or abductors (*a.b.m.*<sup>9</sup>) arise from the entapophyses of the preceding metamere instead of from the haemal side of the carapace; and the muscles which serve to flex and extend the terminal portions of the appendage are more numerous and better developed.

#### 4. DIGESTIVE SYSTEM.

The mouth (Text-fig. 2; Pl. VIII, Fig. 3, *m.*) is situated nearly in the center of the neural side of the cephalothorax, and is surrounded by the chelicerae, five pairs of mandibles, and the



chilaria. The mandibles and chelae of the second, third, fourth, and fifth pairs of legs, aided by the chelicerae, tear the food to pieces and cram it into the mouth. The mandibles of the sixth pair of legs crush the hard portions, and the chilaria serve to push the food forward, within reach of the mandibles.

The *oesophagus* (Pls. VI and VIII, Figs. 1, 3, and 4, *oe.*) passes through the circum-oesophageal collar, turns anteriorly neural to the endocranium, and runs forward to the muscular stomach or proventriculus (Pls. VI, VIII, and IX, Figs. 1, 3-5, *prov.*), which lies at the anterior extremity of the cephalothorax.

The *proventriculus* (Pls. VI, VIII, and IX, Figs. 1, 3-5, *prov.*) is *V*-shaped, and the haemal arm communicates with the intestine by a pyloric valve, which appears as a large muscular papilla at the anterior end of the intestine. The walls of the oesophagus, proventriculus, and pyloric valve are very muscular, and are lined with chitin, which is thrown into longitudinal ridges or rugae.

The *intestine* (Text-figs. 2-4; Pls. VI, VIII, and IX, Figs. 1, 3, 4, and 6, *int.*) is a straight tube running posteriorly from the proventriculus, haemal to the endocranium and ventral cord, and neural to the heart. Its posterior extremity passes into a short rectum or proctodaeum (Pl. VIII, Figs. 3 and 4, *proc.*). Anteriorly the intestine is large, but decreases in size posteriorly. The larger anterior portion receives two pairs of hepatic ducts (Pl. VIII, Fig. 3, *h.d.<sup>a-b</sup>*), which enter at the sides of the intestine nearly opposite the mouth. The walls of the intestine are supplied with both longitudinal and circular muscle fibers, but they are much thinner than the walls of the other portions of the alimentary canal.

The *rectum or proctodaeum* (Pl. VIII, Figs. 3 and 4, *proc.*) is a short tube passing from the intestine to the anus. It is lined like the oesophagus with chitinous rugae, and its walls are supplied with well-developed muscles for the ejection of faeces.

The *anus* (Pls. VI and VIII, Figs. 1 and 3, *a.*) is a longitudinal slit capable of being opened and closed by the anal muscles already described.

The *liver* consists of a great mass of tubules ramifying over

a large portion of the cephalothorax and abdomen. These communicate with ducts which are collected into the two pairs of large hepatic ducts (*h.d.<sup>a-b</sup>*) entering the intestine.

## 5. THE CIRCULATORY SYSTEM.

The circulatory system has been worked out very accurately by A. Milne-Edwards, but some structures have been overlooked in connection with the heart. This organ will, therefore, be taken up in some detail.

### a. *The Heart* (Text-figs. 2-4; Pls. VIII and IX, Figs. 3, 5-8, *ht.*).

The heart, which is very large in *Limulus* in comparison with the size of the body, lies on the haemal side of the animal, directly beneath the carapace and haemal to the intestine. It extends from a point midway between the lateral eyes back to about the middle of the abdomen, being fully one-half as long as the body exclusive of the caudal spine. It has the general appearance of a jointed tube, and attains a length of about five inches in the adult male and about six inches in the female, with a diameter of from half to three-quarters of an inch. Longitudinal strands of connective tissue give it a striated appearance, and a large median ganglionated nerve (Text-figs. 3 and 4; Pls. VIII-X, Figs. 3, 5, 6, 8-10, *m.c.n.*) is very conspicuous upon the haemal side. In cross-section it is somewhat triangular in the middle portion, but is flattened haemo-neurally toward the extremities. The largest portion is just back of the middle, and from this point it tapers in both directions.

There are eight pairs of transverse slit-like ostia (Pls. VIII-X, Figs. 3, 5, 6, 8, and 9, *os.<sup>b-13</sup>*) upon the haemal side of the heart, partially concealed by a grating of longitudinal connective-tissue strands lying across the openings. A ninth pair of rudimentary ostia (Pl. IX, Fig. 8, *r.os.*) are discernible at the anterior extremity. They appear as two shallow pits on the inner surface of the haemal wall of the heart, just behind the aortic arches (*ao.a.*) and in front of the aortic valve (*a.v.*).

Around the heart is a large pericardial sinus (Text-figs. 2-4; Pls. VIII and IX, Figs. 3, 5, and 6, *p.s.*), enclosed by a membranous pericardium (*p.*). Upon the neural side this membrane is well defined, stretching across the body, between the heart and the intestine. At the sides it is attached, in the abdominal region, to the entapophyses, to the bases of the branchial cartilages, and to the haemal carapace between the successive entapophyses. In the cephalothorax it is attached to the first pair of entapophyses, and to the carapace outside of the origins of the inter-tergal muscles. Posteriorly and anteriorly the pericardium is continuous with the neural walls of the heart.

Upon the haemal side of the pericardial space the pericardium, as such, does not exist, or, at least, is indistinguishable from the epidermis. A considerable amount of areolar tissue fills the haemal side, and many of the interstices of the pericardial sinus.

Eight pairs of rather broad bands of connective tissue, the alary muscles (Pl. IX, Figs. 5 and 6, *al.m.*<sup>6-13</sup>) of Van der Hoeven, spring from the lateral edges of the heart, opposite the ostia, and fuse at their distal ends with the pericardium, forming a strong lateral support for the heart. Those in the abdominal region enter the venous canals opening into the pericardial sinus.

Neurally the heart is attached to the pericardium throughout its entire length by numerous connective-tissue fibers. Haemally it is suspended from the carapace, opposite each pair of ostia, by small strands of connective tissue which are continuous with the longitudinal fibers of the heart. From the anterior extremity of the heart, opposite the rudimentary ostia, a pair of tendinous bands (*al.m.s.*), comparable to a pair of alary muscles, run forward and upward a short distance beyond the limits of the pericardium, and attach themselves to the carapace close to the insertions of the tergo-proplastral muscles (Pl. IX, Fig. 5, *t.p.m.s.*). At the posterior end a sheet of connective tissue attaches the extremity of the heart to the carapace.

The pericardial sinus (Text-figs. 2-4; Pls. VIII and IX, Figs. 3, 5, and 6, *p.s.*) surrounds the heart from the extreme posterior end to a point about opposite the rudimentary ostia.

The posterior portion receives the five pairs of canals (*b.c.c.*<sup>9-13</sup>) from the gills.

In normal specimens there are eleven arteries given off from the heart, three from the anterior extremity, and four pairs from the sides, opposite the four anterior pairs of functional ostia. The two large anterior arteries, *aortic arches* (Pls. VIII and IX, Figs. 3, 5, and 8, *ao.a.*), curve downward, one on each side of the proventriculus, to the circum-oesophageal collar. The median artery, *arteria frontalis* (*f.ar.*), goes directly forward over the haemal surface of the proventriculus. A large pocket-shaped valve (Pl. IX, Fig. 8, *a.v.*), much like a vertebrate semilunar valve, lies upon the haemal wall of the heart at the base of the aortic arches, and just behind the rudimentary ostia. It prevents a backward flow of the blood from the aortic arches and from the *arteria frontalis*.

The lateral arteries, *arteriae laterales* (Pls. VIII-X, Figs. 3, 5, 8, and 9, *l.ar.*<sup>6-9</sup>), arise from the lateral, neural corners of the heart, directly beneath the four anterior pairs of functional ostia (*os.*<sup>6-9</sup>), and pass downward into the pericardium, and outward to a pair of longitudinal collecting arteries, the *arteriae*

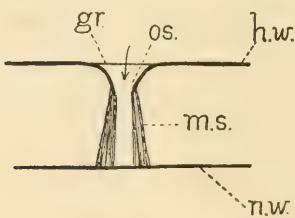


FIG. 7. — Diagram showing the mechanism of the valves of the ostia. *h.w.*, haemal wall of heart; *n.w.*, neural wall of heart; *os.*, ostium of heart; *gr.*, grating of elastic fibers upon the outside of the heart, bridging over the ostium, and preventing the lips of the ostium from spreading apart; *m.s.*, muscle fibers attached to the lips of the ostia at the outer corners.

*collaterales* (*c.ar.*). The *arteriae collaterales* (Pl. IX, Figs. 5, 6, and 8, *c.ar.*) pass backward, giving off numerous branches, and unite behind the posterior end of the heart to form a median artery, the *arteria abdominalis superior* (*s.a.ar.*), which appears to proceed from the posterior extremity of the heart.

The *arteriae laterales* are supplied with paired semilunar valves (Pl. IX, Fig. 8, *s.v.*<sup>6-9</sup>) at their points of origin from the heart. These valves are upon the posterior and anterior walls of the arteries.

Each of the ostia is also provided with a valve, the action of which is best seen in young *Limuli*. The lips of the ostia (Text-fig. 7, *os.*) turn inward toward the lumen of the heart, and this invagination is greatest at the outer corners,



where a few muscle fibers (*m.s.*) are inserted upon the invaginated lips and attached to the neural wall of the heart. Upon the outside of the heart a grating (*gr.*) of elastic fibers stretches across the ostia and prevents the lips from separating too much. The valves are thus kept from being evaginated by the pressure of the blood when the heart contracts.

*Histology of the Heart.*—A cross-section of the heart shows the cardiac walls to be composed of three layers: (1) a median, dense, connective-tissue, basement membrane (Pl. IX, Fig. 7, *b.mem.*); (2) an outer longitudinal layer of thick elastic fibers (*l.c.s.*); and (3) an inner muscular layer (*a.m.f.*). The muscle fibers, which are distinctly cross-striated, are inserted upon the basement membrane and extend across the lumen of the heart, branching, and anastomosing with each other. They are loosely arranged around a central lumen, and form a circular layer thickest in the lateral angles of the heart. There is no endothelium, and the blood circulates freely around the individual muscle fibers. This muscular layer extends from the extreme posterior limit of the heart to a point on the neural side, opposite the aortic valve. On the haemal side (Pl. IX, Fig. 5) its anterior boundary curves around the posterior end of the aortic valve.

In each angle of the heart is a longitudinal nerve (Text-figs. 2-4; Pls. VIII-X, Figs. 3, 5-10, *m.c.n.* and *l.c.n.*) lying between the elastic fibers, outside of the basement membrane, but enclosed by a sheath which is continuous with the membrane.

#### b. *Arterial System.*

The origins of the arteries from the heart in the normal condition have been described above. There is, however, often considerable variation. The *arteria frontalis* (Pls. VIII and IX, Figs. 3-5 and 8, *f.a.r.*) may arise directly between the two aortic arches, or from either one, or it may be absent entirely. When present it gives off a small branch, which goes posteriorly, haemal to the heart, and supplies the intertergal muscles, areolar tissue, and epidermis in the haemal median line. The main trunk runs forward in the median

line, supplying the tissues on the haemal side of the body in the neighborhood of the proventriculus, and divides at the anterior margin of the carapace to form two *anterior marginal arteries*. These follow the edges of the carapace around to the posterior angles of the cephalothorax, where they are joined by branches from the *arteriae collaterales*.

The *aortic arches* (Pls. VIII and IX, Figs. 3-5 and 8, *ao.a.*) curve downward upon each side of the proventriculus, supply this organ and the oesophagus, and then follow the oesophagus backward to the vascular ring, which encloses the nerve collar. The vascular ring gives off arteries anteriorly to the oesophagus, to the tissues in the median line neural to the oesophagus, and to the median and lateral eyes. The chelicerae, five pairs of ambulatory appendages, chilaria, and operculum also receive large arteries from the vascular ring. The *ventral artery* (Pl. IX, Fig. 6, *v.ar.*), which sheathes the ventral cord, is given off from the posterior side of the ring. The longitudinal abdominal muscles, the neural side of the intestine, and the five pairs of gills receive their blood supply from the ventral artery. Posteriorly this artery divides into a number of branches, some supplying the muscles of the caudal spine, but the main branches pass on either side of the rectum to the interior of the caudal spine. A little in front of the anus these arteries give off branches which, after supplying the rectum, pass haemally on each side of the rectum and anastomose with the *arteria abdominalis superior* (*s.a.ar.*).

The origins of the four pairs of lateral arteries (*l.ar.*<sup>6-9</sup>) and their union with the *arteriae collaterales* (*c.ar.*) have already been described. An interesting variation, however, sometimes occurs. A fifth lateral artery (Pl. IX, Fig. 5, *l.ar.*<sup>5</sup>) has been found arising from the base of the left aortic arch, anterior to the aortic valve, and opposite the rudimentary ostia (*r.os.*). This artery has no semilunar valves, but it joins the collateral artery just as do the other lateral arteries. In the same specimen in which this lateral artery was found, a small artery was found in the corresponding position upon the opposite side, but this did not connect with the collateral artery of that side. It supplied the tergo-proplastral muscles.











Anteriorly the collateral arteries give off branches to the tergo-proplastral and tergo-coxal muscles, and to the intestine. Opposite the second pair of ostia a large pair of arteries are given off, which pass outward toward the posterior angles of the cephalothorax and anastomose with the anterior marginal arteries. Midway between the median line and the edge of the carapace a large branch, the hepatic artery, is given off anteriorly, supplying the liver and anastomosing with the lateral eye arteries. At the same point a *posterior marginal artery* is given off. This passes posteriorly along the margin of the abdominal carapace and anastomoses with the superior abdominal artery at the base of the caudal spine.

The posterior portions of the collateral arteries send branches to the intestine, to the external branchial muscles, and to the muscles of the caudal spine. The *arteria abdominalis superior* (*s.a.ar.*) gives off branches to the haemal side of the intestine, anastomoses with branches from the ventral artery and posterior marginal artery, and terminates in the caudal spine. All the arteries divide ultimately into very fine arterioles, which open into venous spaces.

### c. Venous System.

There are no veins with definite walls lined with epithelium. The venous system consists, for the most part, of tubular spaces in the areolar tissue, or irregular spaces between the various organs. The blood is collected from these spaces into a pair of longitudinal sinuses (Pl. IX, Fig. 6, *v.c.s.*) upon the neural side of the body. These sinuses have already been described as spaces, between the branchio-thoracic muscles and the longitudinal abdominal muscles, roofed in on the haemal side by a membrane which furnishes attachment for the veno-pericardiac muscles. From these venous sinuses the blood passes into the operculum and the lamellae of the five pairs of gills. From each gill a large branchio-cardiac canal (Pl. IX, Figs. 5 and 6, *b.c.c.*<sup>9-13</sup>) carries the blood to the pericardial sinus (*p.s.*). A canal (*b.c.c.*<sup>8</sup>) from the operculum unites with the branchio-cardiac canal (*b.c.c.*<sup>9</sup>) of the first gill. There are, therefore,

only five pairs of these canals entering the pericardial sinus, although Milne-Edwards describes six.

From the pericardial sinus the blood enters the heart through eight pairs of functional ostia.

## 6. THE EXCRETORY SYSTEM.

The nephridia, brick-red glands, or coxal glands (Pl. VII, Fig. 2, *n.<sup>2-5</sup>*), as they have been variously called, consist of a mass of tubules in the cephalothorax on each side of the endocranium. An elongated portion lies on the haemal side of the plastro-coxal muscles, and four lobes (*n.<sup>2-5</sup>*) descend from this into the bases of the second, third, fourth, and fifth appendages, respectively. The lobes lie between the slips of the superior plastro-coxal muscles and communicate with each other by ducts on the neural sides of these muscles. A duct leads to the exterior from the lobe in the fifth appendage. The external opening (*n.o.*) is upon the posterior side of the base of the fifth appendage.

## 7. THE REPRODUCTIVE SYSTEM.

*Limulus* is dioecious, and the male can be distinguished by the thicker and subchelate character of the second pair of appendages which are used, during the breeding season, in clinging to the posterior margin of the abdominal carapace of the female. Both the ovary and testes are retiform, the network of tubules, which compose these glands, extending through the cephalothorax and a large part of the abdomen. The paired oviducts and vasa deferentia have muscular walls, and open to the exterior by apertures at the summits of two genital papillae upon the posterior surface of the base of the operculum.

## II. THE NERVOUS SYSTEM.

As has already been said, we are indebted for our knowledge of the nervous system of *Limulus* chiefly to Owen and Milne-Edwards, although Packard, Viallanes, and Patten have more



recently devoted some attention to the development and morphology of the brain.

Owen and Milne-Edwards gave a general plan of the distribution of the larger nerves arising from the brain and ventral cord, but did not agree upon the number of these nerves, and left practically untouched the innervation of the heart, intestine, and appendages. Packard and Viallanes confined their investigations principally to the supra-oesophageal ganglion and the nerves arising therefrom, and restricted the term "brain" to this portion of the nervous system.

Patten figured and described the entire circum-oesophageal collar and ventral cord and applied the term "brain" to the whole nerve ring. He furthermore divided the brain into four regions: (1) the fore-brain, which comprises the cerebral lobes or supra-oesophageal ganglion; (2) the mid-brain, or cheliceral neuromere; (3) the hind-brain, or six thoracic neuromeres; and (4) the accessory brain, or chilial and opercular neuromeres. In the following description of the brain the nomenclature of Dr. Patten will be followed.

## I. THE CENTRAL NERVOUS SYSTEM.

The central nervous system may be divided into brain and ventral cord, the former consisting of the fused ganglia of the circum-oesophageal collar, and the latter of the abdominal ganglia and their longitudinal connectives.

As the primary object of this paper is to give a clear presentation of the distribution of the peripheral nerves, the internal structures of the central nervous system will not be discussed further than is necessary in defining the origins of the various nerves.

### a. *The Brain.*

In the adult *Limulus* the brain (Pls. VI-VIII, and X, Figs. 1-3, 11, and 12) is nearly circular and fits snugly around the oesophagus close to the mouth. It is included within a vascular ring and is thus completely bathed in blood. At the sides of the oesophagus there is a slight flexure, in a neural

direction, which throws the cerebral lobes a little downward. One cross-commissure (*a.c.*) anterior to the mouth and four (*p.o.c.*<sup>2-5</sup>) posterior to the mouth can be seen, from the exterior. Numerous nerves radiate from the sides of the brain. Those going to the appendages bend neurally, giving to the brain a concave appearance upon the neural side and a convex appearance upon the haemal side.

(1) *The Fore-Brain.* — The fore-brain (*f.br.*) lies entirely in front of the mouth, and, when the arterial sheath is removed, appears as two convoluted lobes, the cerebral lobes, separated upon the neural side by a deep longitudinal fissure. Upon the haemal side the fore-brain is depressed in the middle line between the enlarged bases of the lateral eye nerves (*l.e.n.*).

From the semicircular lobes lying in this depression the median eye nerve (*m.ey.n.*) arises by four roots.

The median olfactory nerves (*ol.n.*) arise from the anterior extremity of the fore-brain, the lateral ones from the middle lobes of the optic ganglia.

(2) *The Mid-Brain or Tween-Brain.* — This is represented by the cheliceral or first thoracic neuromere. A typical neuromere (Text-fig. 9) in *Limulus* consists, according to Patten, of a pair of ganglia united across the median line by several cross-commissures (*c.c.*); two pairs of nerves, a neural pair (*n.n.*) supplying the appendages, and a haemal pair (*h.n.*) supplying the internal organs and the lateral expansions of the carapace.

The neural nerves (Text-figs. 1 and 13; Pls. VI–VIII and X, Figs. 1–3, 11, and 12, *n.n.*) arise from the neural side of the brain posterior to the cerebral lobes and pass neurally to the chelicerae. Some small nerves (Text-fig. 1, *e.p.n.*), arising near the bases of the neural nerves, supply the tergo-coxal muscles of the chelicerae.

The haemal nerves (Pl. X, Figs. 11 and 12, *h.n.*) arise from the haemal side of the brain just posterior to the origins of the lateral eye nerves (*l.e.n.*), and present a very exceptional distribution. After fusing with the haemal nerves of the second neuromere they separate again, and, curving around posteriorly, innervate the epidermis upon the neural side of the body out-

side the bases of the appendages, and extend far back upon the abdomen.

The pre-oral cross-commissure (*a.c.*), which belongs to this neuromere, is more neural in position than the post-oral ones, and is also characterized by the fact that it gives off three nerves (*la.n.*) to the rostrum, or upper lip.

In addition to these peculiarities the cheliceral neuromere bears upon its inner side a pair of stomodaeal ganglia and a pair of stomodaeal nerves (*st.n.*) which supply the oesophagus and proventriculus.

(3) *The Hind-Brain.*—The hind-brain consists of five thoracic neuromeres, the second to the sixth inclusive. Each neuromere (Text-fig. 9) consists of a pair of ganglia united by cross-commissures, and two pairs of nerves, a neural pair (*n.n.*) supplying the appendages, and a haemal pair (*h.n.*) supplying internal organs and the lateral expansions of the carapace. The mandibular nerves (*m.n.*) arise from the bases of the neural nerves upon the neural side, and the ento-coxal nerves, three in number (*a.e.n.*, *p.e.n.*, and *m.e.n.*), upon the haemal side of the same nerves.

A pair of nerves (Text-fig. 13; Pl. X, Figs. 11 and 12, *i.n.*<sup>2</sup>), which resemble in their distribution the intestinal nerves, arise from the haemal sides of the bases of the haemal nerves (*h.n.*<sup>2</sup>) of the second thoracic neuromere. In the third, fourth, and fifth neuromeres the intestinal branches are absent; in the sixth they are again present (*i.n.*<sup>6</sup>), but at some distance from the bases of the haemal nerves (*h.n.*<sup>6</sup>).

In the sixth neuromere a cardiac branch (Text-figs. 3 and 9; Pls. VI–IX, Figs. 1–3 and 5, *s.c.n.*<sup>6</sup>) arises from the haemal nerve still farther out than the origin of the intestinal nerve. A small nerve (Pls. VI and VII, Figs. 1 and 2, *x.*), which could not be traced out, was found arising from the haemal nerve (*h.n.*<sup>5</sup>) of the fifth neuromere, and apparently corresponding to the cardiac nerves of other segments.

(4) *The Accessory Brain.*—This contains two neuromeres, the chilarial and opercular neuromeres, which are fused with each other and form the posterior side of the circum-oesophageal collar. Each contains the usual number of elements of the typical neuromere.

The chilarial and opercular nerves (Pls. VI–VIII and X, Figs. 1–3, 11, and 12, *n.n.*<sup>7</sup> and *n.n.*<sup>8</sup>) arise from the neural side of this portion of the brain and pass backward through the occipital ring to their respective appendages. Mandibular nerves are absent, and the ento-coxal nerves are so modified as not to be recognizable as such.

The haemal nerves (*h.n.*<sup>7</sup> and *h.n.*<sup>8</sup>) arise from the haemal side of the brain and pass backward through the occipital ring and outward to the sides of the carapace. Intestinal branches (*i.n.*<sup>7</sup> and *i.n.*<sup>8</sup>) arise at some distance from the bases of the haemal nerves, and cardiac branches (*s.c.n.*<sup>7</sup> and *s.c.n.*<sup>8</sup>) arise still farther out.

It is worthy of notice that the cardiac branches of these two neuromeres fuse with each other, give a recurrent branch to the lateral sympathetic, and send a pericardial branch, posteriorly, in the pericardium; the latter branch also gives recurrent branches to the cardiac nerves of the five branchial neuromeres. The fusion of sympathetic nerves in this region is of the utmost importance, as it supports in a most satisfactory manner the suggestion of Dr. Patten that we have here, in both scorpions and *Limulus*, the beginnings of a vagus region.

The neural and haemal nerves of the accessory brain, together with the ventral cord, pass through the occipital ring.

#### b. *The Ventral Cord.*

This portion of the central nervous system consists of five paired branchial ganglia and three paired post-branchial ganglia (Text-figs. 6 and 18, *a.g.*<sup>9-16</sup>), united by two longitudinal connectives. The double nature of the ventral cord is not apparent unless the ensheathing artery is removed.

The first five branchial ganglia (*a.g.*<sup>9-13</sup>) are separate, but the three post-branchial ganglia (*a.g.*<sup>14-16</sup>) are intimately united with one another. The first three abdominal ganglia lie just in front of the corresponding abdominal endochondrites (Text-fig. 6, *a.e.*<sup>9</sup>; Pls. VI and VIII, Figs. 1, 3, and 4, *a.e.*<sup>9-11</sup>); the fourth and fifth (*a.g.*<sup>12-13</sup>) lie in front of the next endochondrite (*a.e.*<sup>12</sup>); the three fused terminal ganglia (*a.g.*<sup>14-16</sup>) lie considerably in



front of the last endochondrite (*a.e.<sup>13</sup>*), which belongs to the fifth branchial metamere.

The five branchial neuromeres are typical neuromeres, each with its double ganglion and cross-commissure, and neural and haemal pairs of nerves (Text-fig. 8). The neural nerves (Text-figs. 6, 8, and 12; Pls. VI, VIII, and IX, Figs. 1, 3, 4, and 6, *n.n.<sup>9-13</sup>*) arise from the posterior end of the ganglion and give off intestinal branches (*i.n.<sup>9-13</sup>*) close to their origins. The cardiac branches (*s.c.n.<sup>9-13</sup>*) communicate with the lateral sympathetic (*l.s.n.*) and with the pericardial nerve (*p.n.*) by recurrent branches.

The post-branchial neuromeres are not well defined. The ganglia are closely fused together, and the neural nerves are absent.

The haemal nerves (*h.n.<sup>14-16</sup>*) of all three neuromeres give off intestinal branches (*i.n.<sup>14-16</sup>*), and post-cardiac branches (*s.c.n.<sup>14-15</sup>*), which have a distribution similar to the cardiac branches, are given off from the first two post-branchial nerves. However, as the heart does not extend into the post-branchial metameres, the post-cardiac nerves do not communicate with this organ.

## 2. PERIPHERAL NERVOUS SYSTEM.

### a. *Nerves from the Fore-Brain.*

Six nerves have been found arising from the fore-brain, three olfactory, one median eye, and two lateral eye nerves. These are all accompanied by blood vessels, but there are other blood vessels given off from the same region, which do not accompany nerves and which might easily be mistaken for nerves in a hasty dissection. This fact probably accounts for the varied number of nerves found by different investigators.

(1) *Olfactory Nerves.*—Owen, Milne-Edwards, and Packard have described two integumentary nerves arising from the anterior side of the fore-brain. Patten described three nerves, and showed that they had a most remarkable development and that they supplied an aggregation of sense buds on the under surface of the carapace in the median line, anterior to

the chelicerae. He regarded these buds as probably olfactory in function and called the nerves supplying them the olfactory nerves.

The median olfactory nerve (Pls. VI–VIII, Figs. 1–3, *m.ol.n.*, *ol.n.*) arises from the anterior extremity of the fore-brain in the median line, by two roots, one from each of the cerebral lobes, and passes directly forward beneath the skin to the olfactory organs (*ol.or.*). The proximal end is composed of a mixture of nerve fibers and small ganglion cells which arise, according to Patten, as early outgrowths of the cerebral hemispheres. "The distal end divides into many diverging branches, which can be followed by means of a hand lens to the posterior edge of the olfactory organ; they there begin to anastomose, and form a dense plexus underlying the olfactory region, but a considerable number of fibers extend beyond the olfactory region to the neighboring ectoderm. The lateral olfactory nerves (Pls. VI–VIII, Figs. 1–3, *ol.n.*, *r.ol.n.*, *l.ol.n.*) arise apparently from the anterior part of the brain, but in sections one can follow their roots to the ventral surface into the middle lobe of the optic ganglia. In the adult the proximal ends of the nerves consist of coarse transparent nerve tubes and masses of very large ganglion cells. Their distal extremities also contain many clusters of large ganglion cells. The nerve terminates abruptly just beneath the cuticle on the lateral edge of the olfactory organ. The lateral olfactory nerve is accompanied by a large blood vessel that divides into numerous branches, supplying the tissues in front of the olfactory organ; small nerve filaments accompany some of these blood vessels, and probably supply the ectoderm in the same region. Some larger nerve branches leave the median border of the lateral nerves a little distance back of the olfactory organ, and mingle with the plexus formed by the median nerve" (Patten).

(2) *Median Eye Nerves.*—Owen described two median eye nerves, but other authorities have found but one. The median eye nerve (Pls. VI–IX, Figs. 1–3 and 5, *m.ey.n.*) apparently arises from the anterior border of the fore-brain, but examination of sections shows that it arises by four roots from the semicircular lobes upon the haemal side of the fore-brain. It



passes forward neural to the oesophagus and a little to the right of the median line; turns haemally to the right of the proven-triculus and passes to the median eye upon the haemal surface of the carapace in the median line.

According to Dr. Patten the median eye consists of two ectoparietal and one endoparietal eye, and the endoparietal eye is formed by the fusion of a pair of retinas. He finds that "the distal end (of the median eye nerve) splits up into four branches, two of which plunge directly into the median diverticulum or endoparietal eye, and the other two pass to the paired retinas of the ectoparietal eyes."

(3) *Lateral Eye Nerves*. — The lateral eye nerves (Pls. VI and VIII, Figs. 1-3, *l.e.n.*) arise from the large optic ganglia upon the haemal side of the cerebral lobes, pass forward median to the tergo-coxal muscles of the chelicerae, turn outward around the anterior outer corners of the entocoxites of the second pair of appendages, and then pass backward to the lateral eyes. The distal extremity breaks up into two small branches and a large one. The large one passes directly to the retina of the lateral eye; the two smaller ones pass farther backward to a pigmented body beneath the retina.

A large blood vessel encloses the lateral eye nerve for the greater part of its course, and is continued beyond the lateral eye where it anastomoses with the hepatic artery.

#### DESCRIPTION OF A TYPICAL NEUROMERE.

The nerves of all the neuromeres posterior to the fore-brain conform more or less closely in their distribution to a common plan, which it will be well to keep in mind as we take up the successive neuromeres.

(1) *Abdominal Neuromere*. — The most primitive, and hence the most typical neuromeres are those in the abdominal region, *viz.*, those from the ninth to the thirteenth. In these neuromeres (Text-fig. 8) we have a pair of ganglia (*a.g.*) united by cross-commissures, and also partially fused in the median line. Posteriorly, a pair of neural nerves arise, and, anteriorly, a pair of haemal ones. The neural nerves (*n.n.*)

supply the appendages, and the haemal nerves (*h.n.*) supply the body portion of the metamere.

Close to the ganglion each haemal nerve gives off two small nerves; one of these joins a plexus supplying the longitudinal abdominal muscles, the other divides into two portions, the first going to a haemo-neural muscle, and the second to the intestine.

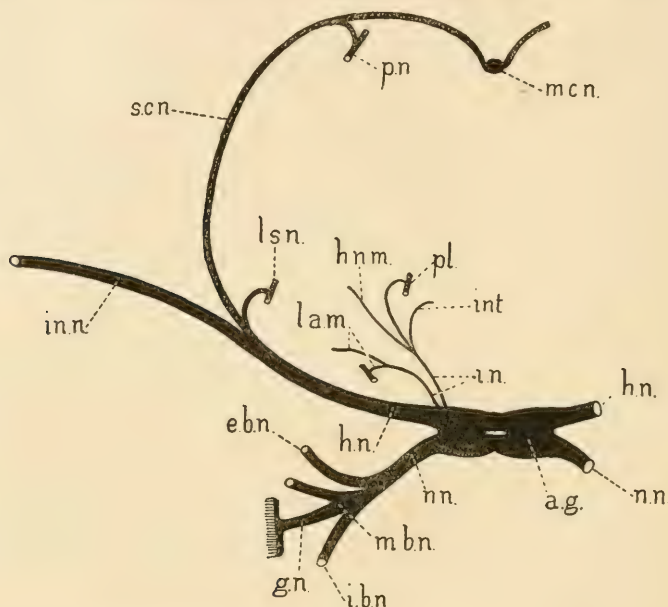


FIG. 8. — Diagram of typical abdominal neuromere.

*a.g.*, abdominal ganglion; *e.b.n.*, external branchial nerve; *g.n.*, gill nerve; *h.n.*, haemal nerve; *i.b.n.*, internal branchial nerve; *i.n.*, intestinal nerve; *i.n.n.*, integumentary nerve; *l.s.n.*, lateral sympathetic nerve; *m.c.n.*, median cardiac nerve; *n.n.*, neural nerve; *p.n.*, pericardial nerve; *s.c.n.*, segmental cardiac nerve; *h.n.m.*, nerve to haemo-neural muscle; *int.*, nerve to intestine; *l.a.m.*, nerve to longitudinal abdominal muscles; *p.l.*, nerve to plexus in tissues surrounding the intestine.

The intestinal portion generally communicates, outside of the intestine, with a plexus which unites the corresponding nerves of successive neuromeres.

At some distance from the ganglion the haemal nerve sends haemally a cardiac nerve (*s.c.n.*). This communicates by a recurrent branch with the lateral sympathetic (*l.s.n.*) which supplies the branchio-thoracic muscles. Another recurrent branch of the cardiac nerve joins the pericardial nerve (*p.n.*),

which runs longitudinally in the areolar tissue alongside the heart. The distal end of the cardiac nerve communicates with the median nerve (*m.c.n.*) of the heart.

The main or integumentary branch (*in.n.*) of the haemal nerve passes outward into the lateral expansions of the carapace.

(2) *The Cephalic or Cranial Neuromere.*—In the cephalothorax the typical neuromere (Fig. 9) is somewhat modified. The

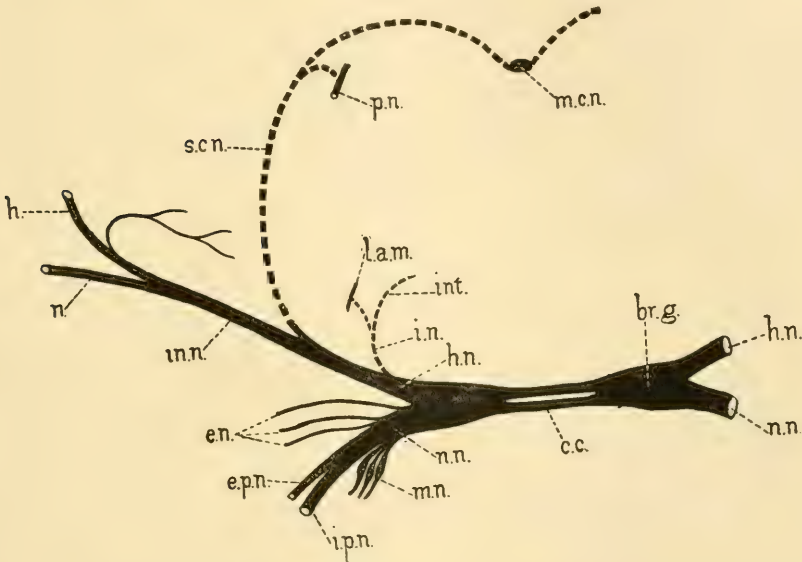


FIG. 9. — Diagram of a typical cranial neuromere.

*br.g.*, ganglion forming part of the brain; *c.c.*, cross-commissures; *e.n.*, ento-coxal nerves; *e.p.n.*, external pedal nerve; *h.*, haemal branch of integumentary nerve; *h.n.*, haemal nerve; *i.n.*, intestinal nerve; *in.n.*, integumentary nerve; *i.p.n.*, internal pedal nerve; *m.n.*, mandibular nerves; *m.c.n.*, median cardiac nerve; *n.*, neural branch of integumentary nerve; *n.n.*, neural nerve; *p.n.*, pericardial nerve; *s.c.n.*, segmental cardiac nerve; *int.*, nerve to intestine; *l.a.m.*, nerve to longitudinal abdominal muscles.

two ganglia are more or less separated, and the cross-commissures are in some cases very long.

The ganglia of the successive neuromeres are crowded together, so that the neural and haemal nerves appear to arise from the neural and haemal sides, respectively, of the ganglia, instead of from the posterior and anterior sides.

The neural nerve (*n.n.*) arises from an enormous ganglion, which in the adult is much obscured by the thick membranes

that surround it. It divides near the ganglion into the entocoxal (*e.n.*), mandibular (*m.n.*), and pedal branches. The entocoxal branches, three in number, supply the muscles inserted upon the entocoxite, and numerous sense organs in the entocoxite itself; the mandibular branch supplies the mandibles; and the pedal branches the remainder of the appendage. In the coxopodite the main pedal branch divides into an external (*e.p.n.*) and an internal portion (*i.p.n.*).

The thoracic haemal nerve, like the abdominal one, has an intestinal, a cardiac, and an integumentary branch. The intestinal branch (*i.n.*) arises at some distance from the brain; the cardiac branch (*s.c.n.*) does not communicate with the lateral sympathetic, and its communication with the heart is doubtful; the integumentary nerve divides into a haemal and a neural portion (*h.* and *n.*).

#### b. *Nerves from the Mid-Brain* (Text-fig. 10).

The mid-brain region contains in addition to the nerves and neuromere of the cheliceral segment a pair of stomodaeal nerves and ganglia and three rostral nerves, arising from the pre-stomodaeal commissure.

(1) *The Neural Nerves.*—The neural nerves (Text-fig. 10; Pls. VI–VIII and X, Figs. 1–3, 11, and 12, *n.n.*<sup>1</sup>) arise from ganglionic swellings situated just back of the cerebral lobes, and pass directly to the inside of the chelicerae.

At the base of the neural nerve arises one or more external pedal nerves (*e.p.n.*). They innervate the tergo-coxal muscles (*e.*<sup>1</sup>, *f.*<sup>1</sup>, *l.m.*<sup>1</sup>), and then pass into the chelicera outside of the main nerve (*i.p.n.*), giving branches to the extensor muscle (*e.*<sup>2</sup>) of the second joint, and ending in the epidermis upon the outer side of the joint. The branches supplying the tergo-coxal muscles represent the ento-coxal branches of a typical neuromere.

The main cheliceral nerve (*i.p.n.*) innervates the epidermis in the first, and the flexor muscle (*f.*<sup>2</sup>) of the second joint. Here it gives branches to the skin and to the muscles (*e.*<sup>3</sup> and *f.*<sup>3</sup>) which move the third joint, and then divides into two large sensory branches which break up in the chelae to supply the











gustatory organs. A large blood vessel accompanies the cheliceral nerve and supplies the appendage with blood.

(2) *The Haemal Nerves.*—The non-ganglionated haemal nerves (*l.n.*, *h.n.*<sup>1</sup>) arise from the haemal side of the neuromere. Each nerve passes forward, dorsal to the lateral eye nerve, upon the median side of the tergo-coxal muscles of the chelicerae, and there fuses with the haemal nerve (*h.n.*) of the second thoracic neuromere. It soon leaves it, however, and, passing haemal to this nerve, turns posteriorly around the base of the second appendage, keeping close to the median eye nerve. The rest of its course lies in the epidermis upon the neural surface of the carapace, outside the bases of the appendages. It does not branch much, if any, until it reaches the skin, just posterior to the sixth leg, beneath a sclerite which lies opposite the flabellum. Here it gives off a number of branches which ramify over the skin. The main nerve is continued onto the abdomen, where it branches at regular intervals, sending a small fiber toward the bases of the first five abdominal appendages. Opposite the last appendage it breaks up into numerous filaments, ramifying over a large portion of the skin in that region.

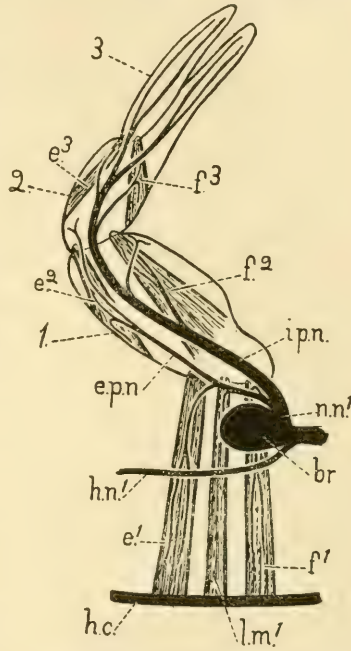


FIG. 10.—Diagram showing muscles and distribution of nerves in chelicera of adult *Limulus*, from the anterior side (natural size). 1, 2, and 3, first, second, and third joints of chelicera; *e.1-3*, extensors of first, second, and third joints, respectively; *f.1-3*, flexors of first, second, and third joints, respectively; *l.m.1*, lateral muscles of first joint; *e.1*, *f.1*, and *l.m.1* constitute the tergo-coxal muscles; *h.c.*, haemal side of the carapace; *br.*, fore-brain; *n.n.1*, neural nerve or cheliceral nerve; *i.p.n.*, internal pedal nerve; *e.p.n.*, external pedal nerve; *h.n.1*, haemal nerve of cheliceral neuromere or lateral nerve.

It is a noteworthy fact that this nerve branches profusely in the skin beneath a sclerite similar to that in the olfactory region, and also that this sclerite and the flabellum, which is highly

sensory, lie opposite each other in a channel through which a continuous current of water passes when the gills are being aerated.

This nerve differs so much in character from the other haemal nerves that it might be considered as an entirely different nerve. Its origin seems to correspond with the origins of the haemal nerve of the typical cranial neuromere, and it passes anterior to the tergo-coxal muscles of the chelicera, just as the haemal nerves of the other neuromeres pass anterior to the tergo-coxal muscles of their own metamere. Its fusion with the second haemal nerve, and its intrusions into all the succeeding metameres without communicating with the haemal nerves, are peculiarities which differentiate it from the other haemal nerves.

It was first described by Owen as a branch of the second haemal nerve, but he did not trace it into the abdominal region. Milne-Edwards overlooked it entirely. Viallanes, in his figure of the brain, figures the root of it and designates it as the recurrent nerve. Patten did not describe its distribution, but in his figure of the brain represented its proximal end as the third pair of haemal nerves.

(3) *The Stomodaeal Nerves.* — The stomodaeal nerves (Pls. VIII and X, Figs. 3, 11, and 12, *st.n.*) were described by Milne-Edwards and by Owen, and called the stomato-gastric nerves. They arise from ganglionic swellings of the nerve collar and extend along each side of the oesophagus, to the proventriculus. Numerous branches are given off to the oesophagus, and sometimes small branches are found arising from the ganglionated bases of the nerves. At the sides of the proventriculus the nerve breaks up into several branches which ramify over the proventriculus, and on to the pyloric valve and the beginning of the intestine. No branches could be traced beyond the pyloric valve.

Milne-Edwards described a ganglion upon each side of the proventriculus, and two very fine branches of the stomato-gastric nerves communicating with the median nerve of the heart, but I have been unable to find either the ganglia or the cardiac branches.

A blood vessel accompanies the stomodaeal nerve and communicates with the aortic arch at the side of the proventriculus, and small vessels from the aortic arch accompany the branches of the stomodaeal nerve over the proventriculus.

(4) *Rostral Nerves*. — Milne-Edwards described two rostral or labral nerves arising from the cerebral lobes. Patten found three, a median and two lateral nerves (Pls. VII, VIII, and X, Figs. 2, 3, 11, and 12, *l.a.n.*), and correctly described them as arising, not from the cerebral lobes, but from the pre-oral commissure. They innervate the rostrum or upper lip.

c. *Nerves from the Hind-Brain* (Text-fig. 11).

The second, third, fourth, fifth, and sixth thoracic neuromeres, which make up the hind-brain, are so much alike that a description of one will, with a few modifications, suffice for all. The third neuromere (Text-fig. 11) is most characteristic and contains the usual elements, a pair of ganglia united by cross-commissures, a pair of neural and a pair of haemal nerves.

(1) *Neural Nerves*. — The neural nerves of the hind-brain arise from ganglionated bases and radiate from the nerve collar to the five pairs of appendages. Owing to the increasing distance from the brain to the base of the appendage, the basal portions of the more posterior nerves are elongated, and the entocoxal and mandibular nerves, which in the anterior neuromeres arise close to the brain, in the sixth neuromere arise at a considerable distance from it (Pl. X, Figs. 11 and 12).

The typical neural nerve (Text-figs. 11, 12, and 8; Pl. X, Figs. 11 and 12, *n.n.*<sup>2-6</sup>) divides, soon after leaving the brain, into three portions, a mandibular portion (*m.n.*) supplying the gustatory organs of the mandibles, a pedal portion (*i.p.n.* and *e.p.n.*) supplying the main portion of the appendage, and three entocoxal branches (*e.n.*) supplying the tergo-coxal muscles and the sensory knobs of the coxopodite.

(a) *Mandibular Branches*. — These nerves were first described by Patten ('93). In the third neuromere there are three mandibular branches (Text-fig. 11, *m.n.*) which arise close together from the neural side of the nerve, not far from the brain. If



the ensheathing artery be removed, the three nerves may be seen arising from a common trunk, which may be traced to the margin of the nerve collar. Each of the three mandibular branches bears at its proximal end a small ganglionic swelling. The first branch gives off a small nerve to the inner mandible

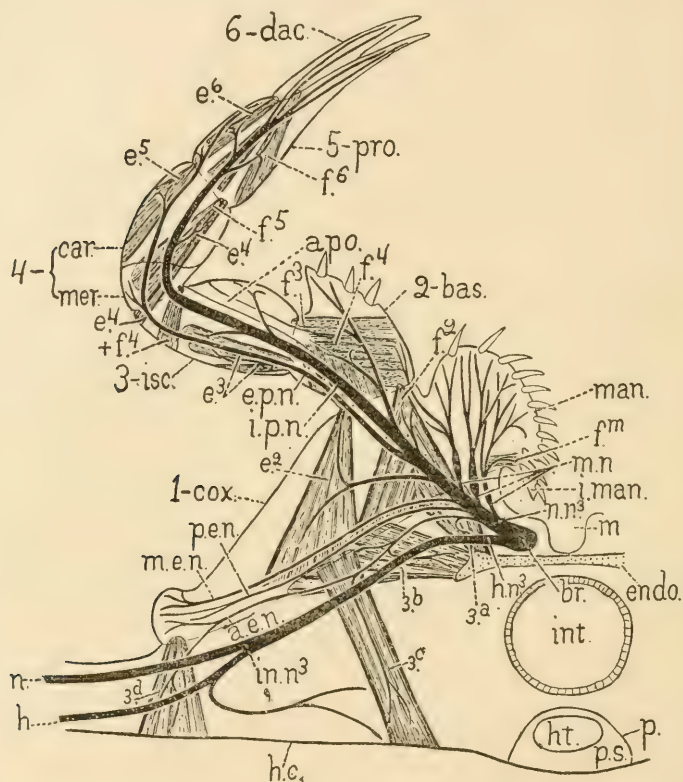


FIG. 11. — Diagram showing muscles and distribution of nerves in the third leg of *Limulus*, from the anterior side ( $\frac{3}{4}$  natural size).

1-cox., coxopodite, or first joint; 2-bas., basipodite, or second joint; 3-isc., ischopodite, or third joint; 4-car., fused carpopodite and meropodite, or fourth joint; 5-pro., propodite, or fifth joint; 6-dac., dactylopodite, or sixth joint; a.p.o., apodeme; h.c., haemal side of carapace; endo., endocranium; ht., heart; int., intestine; i.man., internal mandible; m., mouth; man., mandible; p., pericardium; p.s., pericardial sinus.

MUSCLES: 3a and b, plastro-coxal muscles inserted upon anterior side of entocoxite; 3c and d, tergo-coxal muscles inserted upon anterior side of entocoxite; e.2-6, extensors of second to sixth joints; f.2-6, flexors of second to sixth joints; f.m., flexor of inner mandible.

NERVES: a.e.n., anterior ento-coxal nerve; br., brain; e.p.n., external pedal nerve; h., haemal branch of integumentary nerve; h.n.3, haemal nerve; in.n.3, integumentary branch; i.p.n., internal pedal nerve; m.e.n., median ento-coxal nerve; m.n., mandibular nerves; n., neural branch of integumentary nerve; n.n.3, neural nerve; p.e.n., posterior ento-coxal nerve.



(*i.man.*) and its flexor muscle (*f.<sup>m</sup>*), and then breaks up into fine branches, which ramify over the posterior surface of the mandible (*man.*) and innervate some of the gustatory spines. The second branch supplies the anterior surface of the mandible and the more anterior of the gustatory spines. The third branch innervates the outer portion of the mandible and sends some fine branches to the skin of the inner proximal portion of the basipodite (*2-bas.*).

In the second appendage the first mandibular branch is much reduced in size and innervates only the inner portion of the mandible. There is no inner mandible and, consequently, no corresponding nerve. The second mandibular branch is enlarged and innervates the greater part of the mandible upon both the anterior and posterior sides. The third branch is about as in the third appendage.

In the fourth and fifth appendages there are only two mandibular branches, each with a ganglion near the base. The first one, however, divides into three branches, one to the inner mandible and its flexor muscle, one to the posterior, and one to the anterior side of the mandible. The second ganglionated mandibular branch has a distribution similar to that of the third branch in the third appendage. It is evident that the ultimate distribution of the mandibular branches of these appendages is the same as in the third, but the mode of branching at their bases is a little different.

In the sixth appendage (Text-fig. 12) there are only two mandibular branches (*m.n.*). The first is not ganglionated and is much reduced. It divides into three branches, which are distributed to the inner portion of the mandible, where there are no gustatory spines. The second mandibular branch is ganglionated and corresponds to the third branch in the third appendage. It divides into two portions, one supplying the outer part of the mandible upon which there are a few spines, and the other supplying the inner proximal portion of the basipodite.

(b) *Ento-coxal Branches.*—In the third appendage (Text-figs. 9 and 11; Pl. VII, Fig. 2) there are three ento-coxal branches, an anterior (*a.e.n.*), a posterior (*p.e.n.*), and a median one (*m.e.n.*).

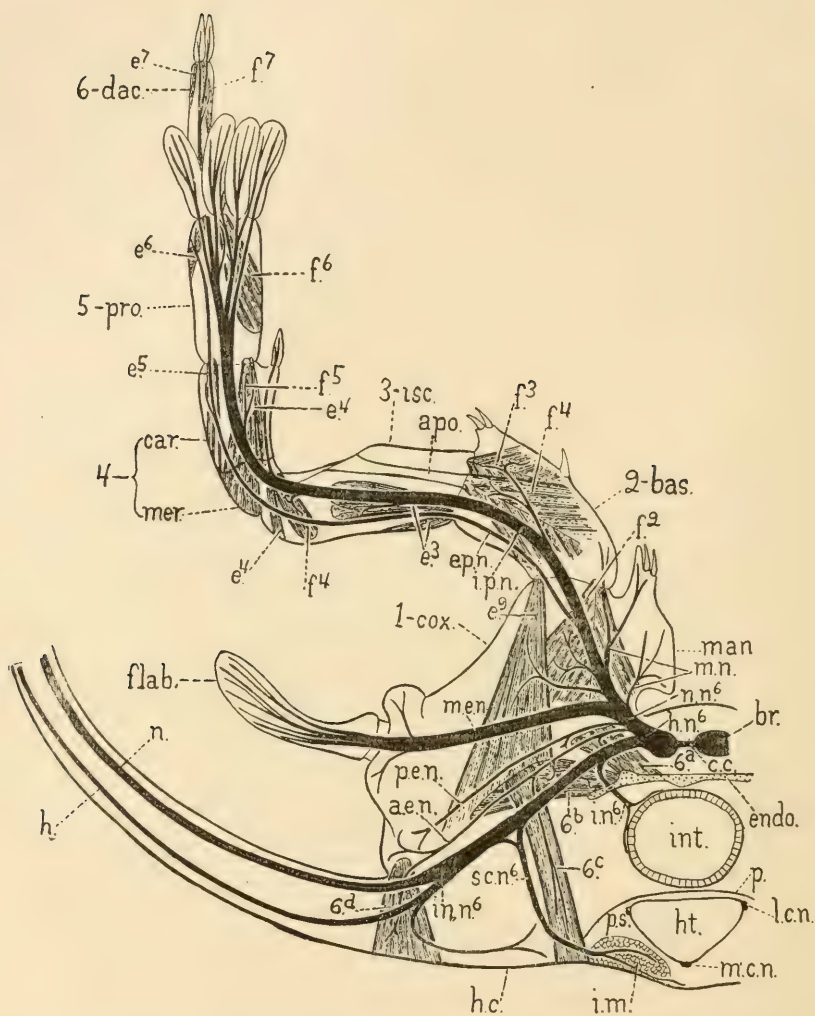


FIG. 12. — Diagram showing the muscles and distribution of the nerves in the sixth leg of *Limulus*, from the anterior side ( $\frac{2}{3}$  natural size).

1-*cox.*, coxopodite, or first joint; 2-*bas.*, basipodite, or second joint; 3-*isc.*, ischiopodite, or third joint; 4-<sup>*car.*</sup><sub>*mer.*</sub>, fused carpopodite and meropodite, or fourth joint; 5-*pro.*, propodite, or fifth joint; 6-*dac.*, dactylopodite, or sixth joint; *apo.*, apodeme; *br.*, brain; *c.c.*, cross-commis-  
sure; *endo.*, endocranium; *flab.*, flabellum; *h.c.*, haemal side of carapace; *ht.*, heart; *int.*,  
intestine; *man.*, mandible; *p.s.*, pericardial sinus.

MUSCLES: *6a* and *b*, plastro-coxal muscles inserted upon anterior side of entocoxite; *6c* and *d*, tergo-coxal muscles inserted upon anterior side of entocoxite; *e.2-7*, extensors of second to seventh joints; *f.2-7*, flexors of second to seventh joints; *i.m.*, inter-tergal muscle.

NERVES: *a.e.n.*, anterior ento-coxal nerve; *e.p.n.*, external pedal nerve; *h.*, haemal branch of integumentary nerve; *h.n.*<sup>3</sup>, haemal nerve; *i.n.*<sup>6</sup>, intestinal nerve; *i.n.*<sup>6</sup>, integumentary branch of haemal nerve; *i.p.n.*, internal pedal nerve; *l.c.n.*, lateral cardiac nerve; *m.c.n.*, median cardiac nerve; *m.e.n.*, median ento-coxal nerve or flabellar nerve; *m.n.*, mandibular nerve; *n.*, neural branch of integumentary nerve; *n.n.*<sup>6</sup>, neural nerve; *p.*, pericardium; *p.e.n.*, posterior ento-coxal nerve; *s.c.n.*<sup>6</sup>, segmental cardiac nerves.

They arise upon the haemal side of the neural nerve, the two former very near the brain, the latter or median one a little farther out. Sometimes there are two additional nerves supplying the inner plastro-coxal muscles, but these may be regarded as branches of the main ento-coxal nerves, for in many specimens these muscles are supplied by nerves which are undoubted branches of the main ento-coxal nerves.

The anterior ento-coxal nerve (*a.e.n.*) arises from the anterior haemal side of the neural nerve quite close to the brain, gives off a small branch to the innermost, anterior, plastro-coxal muscle ( $3^a$ ), then passes down through the substance of the nephridium (Pl. VII, Fig. 2,  $n.^2$ ) and innervates all the muscles ( $3^{b-d}$ ), which are inserted upon the anterior border of the entocoxite, but apparently gives no branches to the nephridium. It terminates in delicate filaments in the areolar tissue at the outer extremity of the entocoxite, and sends some fibers to the anterior sensory knob.

The posterior ento-coxal nerve (*p.e.n.*) arises from the posterior, haemal side of the neural nerve, and innervates all the muscles which are inserted upon the posterior border of the entocoxite. It also passes through the substance of the nephridium, but does not give off any nerves to it. It terminates in areolar tissue at the outer extremity of the entocoxite and innervates the posterior sensory knob.

The median ento-coxal nerve (*m.e.n.*) arises from the haemal side of the neural nerves some distance farther from the brain than the other ento-coxal nerves. It is much smaller than these nerves, and passes out over the surface of the nephridium accompanied by a blood vessel. It is entirely sensory in function and terminates in the median sensory knob and the surrounding tissues.

In the second appendage (Pl. VII, Fig. 2) the anterior and posterior ento-coxal nerves (*a.e.n.*<sup>2</sup> and *p.e.n.*<sup>2</sup>) are similar to those in the third appendage, except that separate nerves supplying the inner plastro-coxal muscles are often present. The median nerve has not yet been found, but it probably exists, and has been overlooked on account of its extreme tenuity. All the median ento-coxal nerves, except the sixth, are very small and difficult to find.

In the fourth and fifth appendages the ento-coxal nerves are essentially the same as in the third appendage.

In the sixth appendage (Text-fig. 12; Pl. VII, Fig. 2) there is an interesting variation. The anterior and posterior ento-coxal nerves (*a.e.n.* and *p.e.n.*) are similar to those in the third appendage, except that they arise at some distance from the brain; but the median ento-coxal nerve (*m.e.n.*) is much enlarged and becomes the flabellar nerve, which breaks up into many filaments inside the flabellum (*flab.*) and supplies the numerous sense buds of this organ. It also gives off a few branches to the epidermis around the base of the flabellum. A large blood vessel accompanies it into the flabellum.

(c) *Pedal Branches.* (See Text-fig. 11). — In the coxopodite (1-cox.) the main pedal nerve gives small branches to the flexor (*f.<sup>2</sup>*) and extensor muscles (*e.<sup>2</sup>*) of the basipodite (2-bas.). A larger branch, the external pedal nerve (*e.p.n.*), leaves the outer side of the pedal nerve and runs parallel to it along the outer side of the leg, through all the joints as far as the distal end of the propodite (5-pro.). This branch is found in all the thoracic appendages, not excepting the chelicerae, and seems to be mainly sensory in function, though it does supply a few muscles.

The main pedal branch, or internal pedal nerve (*i.p.n.*), lies for the most part toward the inner side of the leg between the muscles of the anterior and those of the posterior sides. It gives off both sensory and motor branches all along its course, and supplies all the muscles not supplied by the external pedal nerve. In the second, third, fourth, and fifth legs it terminates in four branches, two to each blade of the chelae.

In the propodite of the sixth leg (Text-fig. 12) it gives off numerous branches to the spatulate organs. The main branch continues into the slender outer joint (6-dac.), and, after supplying the extensor and flexor muscles (*e.<sup>7</sup>* and *f.<sup>7</sup>*) of the terminal chelate portions, divides into two branches which distribute themselves in the two terminal joints.

(2) *Haemal Nerves.* — The typical haemal nerve (Text-fig. 8) consists of three branches, an intestinal (*i.n.*), a cardiac (*s.c.n.*), and an integumentary branch (*in.n.*). In most of the thoracic



neuromeres the intestinal and cardiac branches are absent. The sixth neuromere, however, contains the typical number of branches.

In all cases the haemal nerve (Text-figs. 11 and 12; Pls. VI–VIII, and X, Figs. 1–3, 11, and 12, *h.n.*) arises from the haemal side of the brain slightly anterior to the neural nerve of the same neuromere. It is about half as large as the neural nerve, does not have a ganglionated base, and is not accompanied by an artery except for a short distance from its origin. It is peculiar in having at a greater or less distance from its origin a ganglion-like swelling. This swelling, which has been described by Milne-Edwards, contains, however, no ganglion cells, but the fibers at these points undergo a complicated interlacing, and numerous nuclei are present.

(a) *Intestinal Branches.* — The intestinal branch is absent in the third, fourth, and fifth neuromeres. In the sixth (Text-fig. 12; Pls. VI–VIII and X, Figs. 1–4, 11, and 12, *i.n.*<sup>6</sup>) it arises from the haemal side of the haemal nerve and passes between the plastro-coxal muscles of the fifth and sixth legs, through a foramen (Pl. VIII, Fig. 4, *f.*<sup>6</sup>) in the endochondrite, into the longitudinal abdominal muscles attached to the endochondrite. Here it communicates with a plexus which supplies these muscles. Some of the branches, however, pass on to the intestine. In the third, fourth, and fifth metameres the intestine is supplied by nerves which pass forward from the plexus in the longitudinal abdominal muscles. Some of the nerves have also been traced from this plexus to the tergo-plastral and veno-pericardiac muscles in this region.

In the second metamere a nerve (Text-fig. 13; Pls. VIII and X, Figs. 3, 11, and 12, *i.n.*<sup>2</sup>) is given off from the haemal nerve (*h.n.*<sup>2</sup>) close to its base. It passes haemally upon the median side of the anterior cornu (*a.cor.*), and supplies the tergo-propastral muscles (*t.p.m.*<sup>a and b</sup>). No branch has been observed going to the intestine, but as it supplies muscles similar to those supplied by the other intestinal branches, and also has an origin similar to that of the other intestinal branches, it has been included in the same category.

(b) *Cardiac Branch.* — The cardiac branch (Text-figs. 12

and 9; Pls. VI-X, Figs. 1-3, 5, 11, and 12, *s.c.n.*<sup>6</sup>) is present in the sixth neuromere only. It arises from the haemal side of the haemal nerve (*h.n.*<sup>6</sup>), about midway between the brain and the outer edge of the entocoxite. Where it separates from the haemal nerve a small recurrent branch is sometimes seen passing backward from the cardiac nerve to the integumentary portion (*in.n.*) of the main nerve. It passes

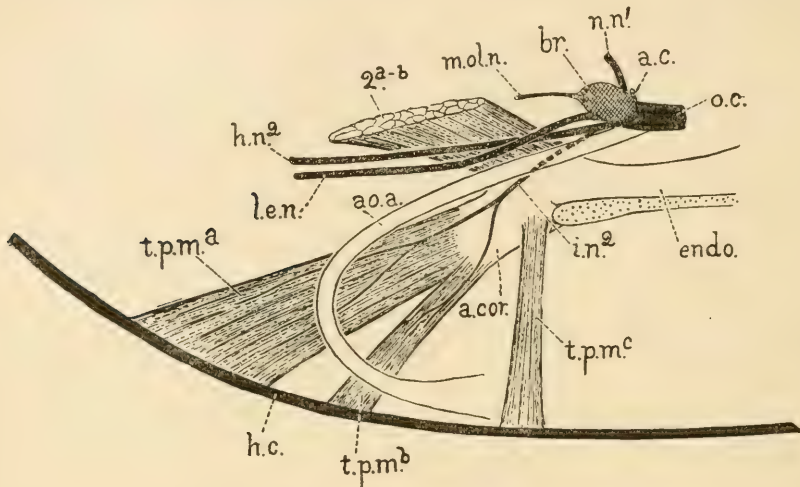


FIG. 13. — Diagram showing the distribution of the intestinal nerve of the second neuromere. The left anterior cornu of the endocranium with attached muscles, one of the aortic arches, and the anterior portion of the brain are represented as seen from the median side. The fore-brain and endocranium are cut in two in the middle line.

*a.cor.*, anterior cornu of the endocranium; *ao.a.*, aortic arch; *endo.*, endocranium; *h.c.*, haemal side of carapace.

MUSCLES: *2a* and *b*, plastro-coxal muscles inserted upon the anterior border of the entocoxite of the second appendage; *t.p.m.a-c*, tergo-proplastral muscles.

NERVES: *a.c.*, anterior commissure; *br.*, fore-brain; *h.n.2*, haemal nerve of second neuromere; *i.n.2*, intestinal nerve of second neuromere; *l.e.n.*, lateral eye nerve; *m.ol.n.*, median olfactory nerve; *n.n.1*, cheliceral nerve; *o.c.*, portion of circum-oesophageal collar.

haemally, outside of the branchio-thoracic muscles (*b.t.m.*) and, bending toward the anterior side, passes through the pericardium to the inter-tergal muscle. Within this muscle its branches anastomose with the cardiac branches of the next posterior neuromere.

Some of the branches supply the epidermis in the haemal median line over the first pair of ostia (*os.*<sup>6</sup>) of the heart, and, although no connection has yet been found between these



branches and the median nerve (*m.e.n.*) of the heart, it is very probable that such a connection exists. The connective-tissue strands supporting the heart in this region are numerous, rendering it very difficult to distinguish nerve fibers and trace them through the mass of other fibers.

In the second, third, and fourth thoracic neuromeres no cardiac branches have been found; in the fifth a small nerve was found which corresponded in origin to the cardiac branches of the other haemal nerves, but its distribution could not be traced out.

(c) *Integumentary Branches.*—The haemal nerve becomes enlarged and flattened near the outer margin of the entocoxite and forms the integumentary nerve (Text-figs. 9, 11, and 12; Pls. VI and VII, Figs. 1 and 2, *in.n.*). It then divides into two main branches, one (*n.*) going to the neural surface of the carapace, and the other (*h.*) to the haemal surface.

The neural branch soon divides into two more branches, and these break up into numerous fibers, which ramify over the neural surface of the carapace and supply the skin, and probably the numerous muscle strands passing between the two surfaces of the lateral expansions of the carapace.

The haemal branch gives off near its origin a small nerve, which turns haemally toward the median line, and supplies the epidermis of the haemal side between the pericardium and the outer edges of the entocoxites. In some cases these branches anastomose in the epidermis with the corresponding branches of the other haemal nerves. The main haemal branch breaks up into small branches which innervate the skin upon the haemal side of the lateral expansions of the carapace.

The second haemal nerve (*h.n.<sup>2</sup>*) is somewhat larger than the others, and its integumentary branches have a larger area of distribution, for they supply the thicker anterior portion of the cephalic shield. It lies almost parallel to the median line, while the sixth one (*h.n.<sup>6</sup>*) lies at right angles to it. The intermediate haemal nerves (*h.n.<sup>3-5</sup>*), owing to the rounded form of the cephalothorax and the central position of the brain, necessarily diverge from each other like the radii of a circle.

Another noticeable feature about the haemal nerves is a

bend near their proximal ends; the proximal end of the second haemal nerve is straight; the third nerve has a slight flexure; the fourth has a greater one; and the fifth and sixth have very marked flexures.

The median eye nerve (*m.e.n.*) passes between the haemal and the neural integumentary branches of the second, third, fourth, and fifth haemal nerves. The first haemal nerve or lateral nerve (*l.n.*) passes haemal to the second haemal nerve and neural to all others.

d. *Nerves from the Accessory Brain* (Text-figs. 14 and 15).

The accessory brain consists of two neuromeres fused together, the seventh, or chilial neuromere and the eighth, or opercular neuromere. Both neuromeres have all the typical elements, but they resemble the abdominal rather than the cranial type. The haemal and neural nerves all arise from the posterior side of the brain, and together with the ventral cord pass through the occipital ring.

(1) *Neural Nerves*.—The neural nerves of the two neuromeres differ so much from each other that it will be necessary to describe them separately.

(a) *Chilial Nerve*.—The paired chilial nerve (Text-fig. 14; Pls. VI–VIII, and X, Figs. 1–3, 11, and 12, *n.n.*<sup>7</sup>) arises from the posterior side of the brain near the median line and neural to the origin of the ventral cord (*v.c.*). It passes posteriorly near the median line close beneath the roof of the occipital ring (*oc.r.*) into the chilium. As it enters the base of the appendage, it gives off branches to all the muscles of the chilium. The main nerve breaks up inside the appendage, supplies the epidermis, and sends a large fiber into each of the gustatory spines which fringe its inner margin.

(b) *Opercular Nerve*.—The opercular nerve (Text-figs. 14 and 15; Pls. VI–VIII, and X, Figs. 1–3, 11, and 12, *n.n.*<sup>8</sup>) arises just posterior to the chilial nerve and passes backwards through the occipital ring near the median line neural to the ventral cord. At the base of the operculum (*op.*<sup>8</sup>) it gives off a small branch to the internal branchial muscle (Text-fig. 12, *i.b.m.*) and then











divides into three main branches, the external, median, and internal, opercular nerves (*e.o.n.*, *m.o.n.*, and *i.o.n.*).

The first of these (*i.o.n.*) again divides into a motor and a sensory branch; the motor branch innervates the large abductor muscles (*ab.m.*<sup>s</sup>) upon the anterior face of the append-

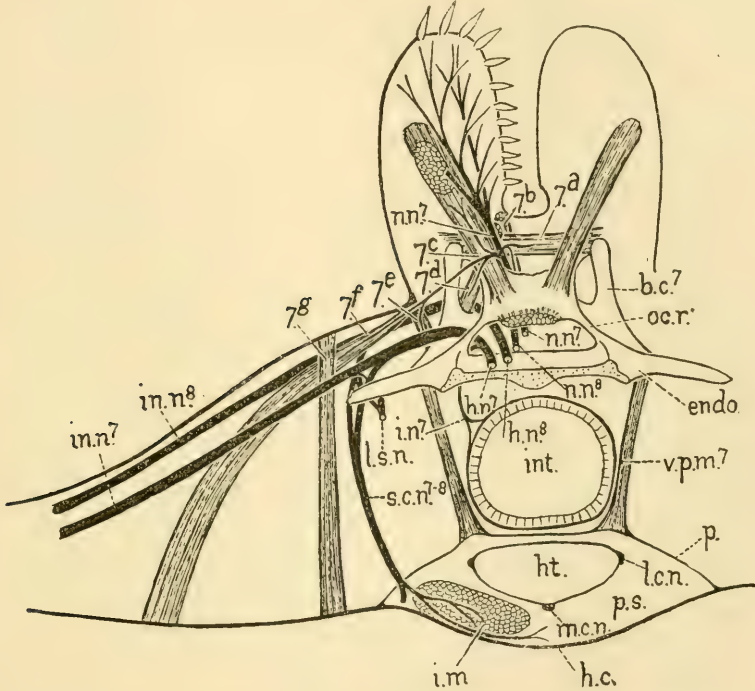


FIG. 14.—Diagram showing the muscles and nerves of the chilaria of *Limulus*, from anterior side. The appendages are revolved outward about 45° (magnified nearly 1½ diameters).

*b.c.7*, capsuliginous bar or branchial cartilage; *endo.*, endocranium; *h.c.*, haemal side of carapace; *ht.*, heart; *int.*, intestine; *oc.r.*, occipital ring; *p.s.*, pericardial sinus.

MUSCLES: *7 a-c*, plastro-coxal muscles; *7 f* and *g*, tergo-coxal muscles; *i.m.*, inter-tergal muscles; *v.p.m.7*, veno-pericardiac muscles.

NERVES: *h.n.7* and *8*, haemal nerves of chilarial and opercular neuromeres; *i.n.7*, intestinal nerve; *in.n.7* and *8*, integumentary branches of haemal nerves of chilarial and opercular segments; *l.c.n.*, lateral cardiac nerve; *l.s.n.*, lateral sympathetic nerve; *m.c.n.*, median cardiac nerve; *n.n.7* and *8*, neural nerves of chilarial and opercular neuromeres; *p.*, pericardium; *s.c.n.7* and *8*, fused segmented cardiac nerves of chilarial and opercular neuromeres.

age, and the sensory branch supplies the epidermis of the anterior face and outer margin of the base of the appendage.

The second branch (*m.o.n.*) also divides into a motor and a sensory branch; the motor branch supplies the external bran-

chial or abductor muscle (*e.b.m.*<sup>8</sup>) upon the posterior face of the appendage; the sensory branch innervates the epidermis of the middle portion of the appendage.

The third branch (*i.o.n.*) is mainly sensory and innervates the distal portions of the appendage. It also contains some motor elements which supply the muscle strands (*o.l.m.* and *i.l.m.*), moving the distal portions (*o.l.* and *i.l.*) of the appendage.

(2) *Haemal Nerves.* — The haemal nerves (Text-figs. 14 and 15; Pls. VI–VIII and X, Figs. 1–3, 11, and 12, *h.n.*<sup>7</sup> and *h.n.*<sup>8</sup>) arise from haemal side of the brain and pass back through the occipital ring and outward to the sides of the body, between the sixth pair of legs and the operculum. The one belonging to the chilial neuromere arises outside of the opercular one and turns outward anterior to the capsuliginous bar, while the other one turns outward posterior to the bar. They are typical haemal nerves and have the usual intestinal, cardiac, and integumentary branches.

(a) *Intestinal Branches.* — The intestinal branch (*i.n.*<sup>7</sup>) of the chilial haemal nerve is given off at the bend in the nerve between the capsuliginous bar (*b.c.*<sup>7</sup>) and the base of the occipital ring, and passes through a foramen (*f.*<sup>7</sup>) in the endocranium to the longitudinal abdominal (*l.a.m.*) muscles and, like the intestinal nerve of the sixth neuromere, communicates with a plexus supplying these muscles and sends a branch to the intestine.

The intestinal branch (*i.n.*<sup>8</sup>) of the opercular haemal nerve is also given off at the bend of the nerve posterior to the capsuliginous bar (*b.c.*<sup>7</sup>). It does not pass through the endocranium but plunges directly into the abdominal muscles. Its distribution is similar to that of the preceding. It is a notable fact that in many cases these intestinal branches have been seen to arise from the haemal nerve by two roots.

(b) *Cardiac Branches.* — The cardiac branches (*s.c.n.*<sup>7</sup> and <sup>8</sup>) of these two neuromeres arise from the haemal nerves at some distance beyond the origins of the intestinal nerves, and fuse together into one large nerve. The opercular root gives off a branch to the lateral sympathetic (*l.s.n.*) which innervates the branchio-thoracic muscles (*b.t.m.*). The fused cardiac nerve

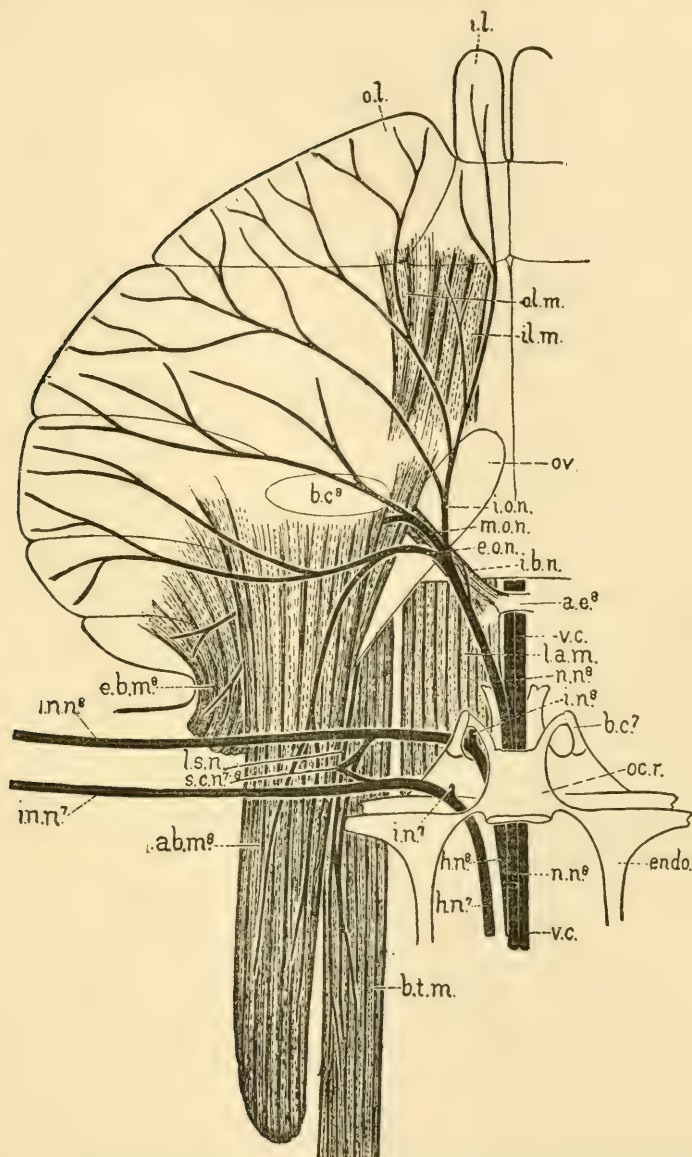


FIG. 15.—Diagram showing the muscles and distribution of the nerves in the operculum. The operculum is flexed upon the abdomen, and is seen from the neural side (about  $1\frac{1}{2}$  natural size). *a.e.*, abdominal endochondrite of opercular segment; *b.c.*, branchial cartilage of operculum; *endo.*, endocranium; *i.l.*, inner lobe of operculum; *o.c.r.*, occipital ring; *o.l.*, outer lobe of operculum; *ov.*, oviduct.

MUSCLES: *a.b.m.*, abductor muscle of operculum; *b.t.m.*, branchio-thoracic muscles; *e.b.m.*, external branchial muscle; *i.b.m.*, internal branchial muscle; *i.l.m.*, muscle of inner lobe; *o.l.m.*, muscle of outer lobe.

NERVES: *e.o.n.*, external branch of opercular nerve; *h.n.* and *8*, haemal nerves of chilial and opercular segments; *i.n.* and *8*, intestinal nerves of chilial and opercular neuromeres; *in.n.* and *8*, integumentary branches of haemal nerves of chilial and opercular neuromeres; *i.o.n.*, internal branch of opercular nerve; *l.s.n.*, lateral sympathetic nerve; *m.o.n.*, median branch of opercular nerve; *n.n.*, neural or opercular nerve; *s.c.n.* and *8*, fused segmental cardiac nerves of the seventh and eighth neuromeres; *v.c.*, ventral cord.

passes haemally outside of the branchio-thoracic muscles, turns toward the median line anterior to the base of the large entapophysis (*enta.*<sup>7 and 8</sup>), and enters the large inter-tergal muscles haemal to the heart. Here it breaks up into anastomosing branches, running forward inside the muscle, and into small fibers which pass toward the median line in the epidermis over the second and third pairs of ostia (*os.*<sup>7 and 8</sup>) of the heart. A large branch, the pericardial nerve (*p.n.*), passes posteriorly in the epidermis haemal to the heart and gives recurrent branches to the cardiac nerves of the gill region.

(c) *Integumentary Branches.* — The integumentary portion of the chilarial nerve (Pl. VI, Fig. 1) supplies a large area of the epidermis on the posterior portion of the cephalothorax, including the posterior angles. A few branches are also distributed to the anterior border of the abdomen. As the lateral expansions of the carapace are thin in this region, haemal and neural branches cannot easily be distinguished, but near the median line we find the usual small haemal branch running in the epidermis haemally and toward the median line.

The integumentary branch of the opercular nerve has a limited area of distribution in the anterior portion of the abdomen. It confines itself to the opercular segment, which is conspicuously marked off by the auricular-shaped processes of the abdominal carapace just posterior to the hinge. A small nerve is given off to the epidermis outside the base of the operculum.

e. *Nerves from the Five Branchial Neuromeres* (Text-figs. 16 and 17).

The nerves from the five branchial neuromeres are very similar in their distribution. These neuromeres are the most typical and primitive, and all the others may be considered as derived from neuromeres similar to them. Each branchial neuromere (Text-fig. 8) contains a pair of fused ganglia (*a.g.*) united by cross-commissures, and a pair of haemal and neural nerves. The neural nerves (*n.n.*) arise from the posterior side of the ganglion and innervate the appendage, and the haemal nerves (*h.n.*) arise from the anterior side and inner-



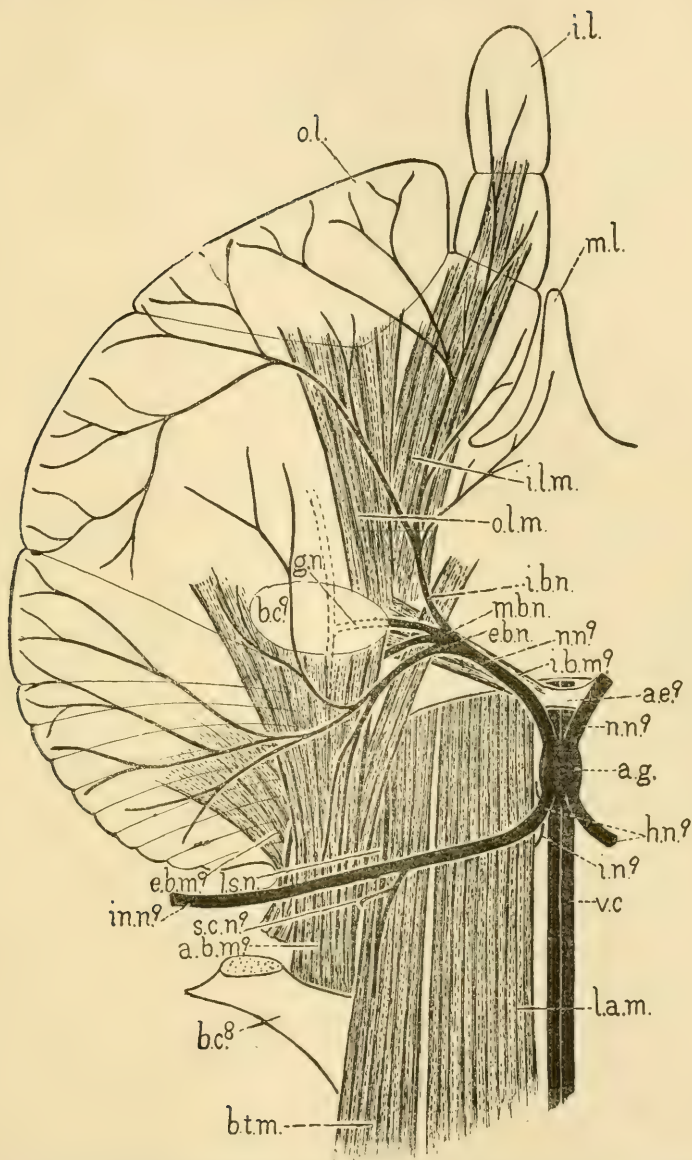


FIG. 16. — Diagram showing the muscles and distribution of nerves in the first gill. The appendage is flexed upon the abdomen, and is seen from the neural side (about  $1\frac{1}{2}$  natural size).

*a.e.9*, abdominal endochondrite; *b.c.8* and *9*, branchial cartilages of operculum and first gill; *i.l.*, inner lobe of gill; *m.l.*, median lobe of gill; *o.l.*, outer lobe of gill.

MUSCLES: *a.b.m.9*, abductor muscle of gill; *b.t.m.*, branchio-thoracic muscles; *e.b.m.9*, external branchial muscle; *i.b.m.9*, internal branchial muscle; *i.l.m.*, inner lobe muscles; *l.a.m.*, longitudinal abdominal muscles; *o.l.m.*, outer lobe muscles.

NERVES: *a.g.*, first abdominal ganglion; *e.b.n.*, external branch of neural nerve; *g.n.*, branch of neural nerve supplying gill book; *i.b.n.*, internal branch of neural nerve; *i.n.9*, intestinal nerve (two branches are shown, a posterior and an anterior one); *in.n.9*, integumentary branch of haemal nerve; *l.s.n.*, lateral sympathetic nerve; *m.b.n.*, median branch of neural nerve; *n.n.9*, neural nerve; *s.c.n.9*, segmental cardiac nerve; *v.c.*, ventral cord.

vate the body portion of the metamere. The haemal nerve is divisible into intestinal (*i.n.*), cardiac (*s.c.n.*), and integumentary branches (*in.n.*), and of these the intestinal and cardiac branches communicate with corresponding branches of other neuromeres by longitudinal connectives.

(1) *Neural or Gill Nerves.* — The neural nerve (Text-figs. 16 and 17, *n.n.*<sup>9</sup>) enters the base of the gill and immediately divides into three branches, the external (*e.b.n.*), median (*m.b.n.*), and internal (*i.b.n.*) branchial nerves.

The external branchial nerve gives a motor branch to the abductor muscles (*ab.m.*<sup>9</sup>) of the anterior face of the gill and a sensory branch to the epidermis of the same region and to the outer portion of the base of the gill.

The median branchial nerve (*m.b.n.*) gives a motor branch to the external branchial or abductor muscle (*e.b.m.*<sup>9</sup>) upon the posterior face of the appendage, and a sensory branch (*g.n.*) to the gill book (Text-fig. 17, *g.b.*). This passes outward posterior to the branchial cartilage (*b.c.*<sup>9</sup>) to the inner edge of the gill book, where it divides into two bundles of fibers which go in opposite directions along the edge of the gill book, and give off a fine nerve fiber to each gill leaf. These fibers follow the margins of the leaves and break up into fine filaments which supply the numerous sense buds in the epidermis.

The internal branchial nerve supplies the muscles (*o.l.m.* and *i.l.m.*) and epidermis of the distal portions (*o.l.* and *i.l.*) of the appendage, and corresponds very closely to the third branch (*i.o.n.*) in the operculum. The median lobe (*m.l.*) of the appendage is also supplied by a branch from the internal branchial nerve.

(2) *Haemal Nerves.* — The haemal nerve (Text-fig. 16; Pls. VI and VIII, Figs. 1 and 3, *h.n.*<sup>9-13</sup>) of the branchial neuromere arises from the anterior end of the abdominal ganglion and passes out over the neural surfaces of the longitudinal abdominal muscles, anterior to the appendage of its own metamere. It divides into three principal branches — (a) intestinal, (b) cardiac, (c) integumentary branches.

(a) *Intestinal Branches.* — In all the gill neuromeres the intestinal branch (*i.n.*<sup>9-13</sup>) arises from the proximal end of the haemal



nerve very close to the ganglion and is either double at its origin or divides, not far from its origin, into two branches. One branch joins the plexus in the longitudinal muscles; the other goes to the intestine and in the majority of cases sends also a branch to the haemo-neural muscle (*h.n.m.*<sup>9-13</sup>) of its own meta-

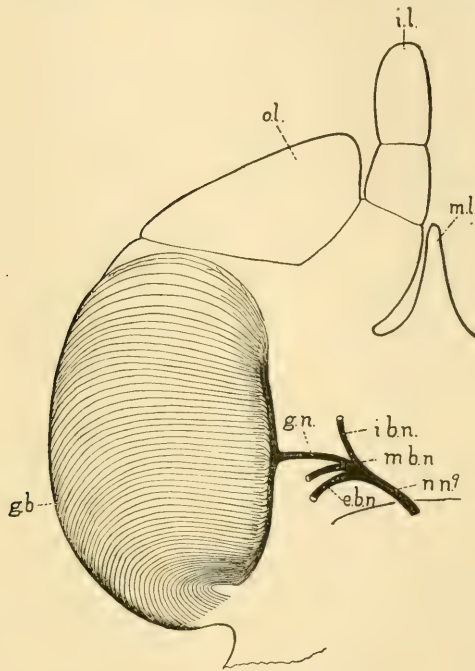


FIG. 17. — Diagram of the first gill, from the posterior side, showing the distribution of the gill nerve to the gill book (about natural size).

*g.b.*, gill book; *i.l.*, inner lobe of the appendage; *m.l.*, median lobe of appendage; *o.l.*, outer lobe of appendage.

NERVES: *e.b.n.*, external branchial nerve; *g.n.*, gill nerve; *i.b.n.*, internal branchial nerve; *m.b.n.*, median branchial nerve; *n.n.9*, neural nerve of the ninth neuromere.

mere. In the last gill neuromere the double nature of the intestinal branch was not found, but as these nerves are all very delicate some of the branches might easily be overlooked in dissection. Sometimes the branch which supplies the intestine goes some distance in the connective tissue, and divides before entering the intestine; and it has been seen to anastomose with the intestinal branches from the other neuromeres.

(b) *The Cardiac Branches.* — The cardiac branch in the

branchial neuromeres is given off (Text-figs. 16 and 18; Pls. VI, VIII, and IX, Figs. 1, 3, 5, and 6, *s.c.n.*<sup>9-13</sup>) outside of the branchio-thoracic muscles (*b.t.m.*) or tendinous stigmata (*t.s.*<sup>9-13</sup>), and, after giving a recurrent branch to the lateral sympathetic (*l.s.n.*), passes haemally, anterior to the entapophysis and posterior to the branchio-cardiac canal (*b.c.c.*<sup>9-13</sup>) of its own neuromere. Having reached the haemal side of the animal, it turns toward the median line, gives a recurrent branch anteriorly to the pericardial nerve (*p.n.*), and breaks up into small branches which ramify through the epidermis, haemal to the heart. Over each pair of ostia (*os.*<sup>9-13</sup>) of the heart a pair of these branches pass down from the epidermis to the median nerve (*m.c.n.*) of the heart.

The cardiac nerve (*s.c.n.*<sup>13</sup>) of the fifth branchial neuromere is a little different from the others. A portion of it sometimes separates from the haemal nerve far back near the base of the latter, and follows it along to the lateral sympathetic, where it receives another branch from the haemal nerve. This latter branch, and not the main cardiac branch, gives the recurrent branch to the lateral sympathetic (Text-fig. 12). Near the entapophysis (*enta.*<sup>13</sup>) it gives a branch to the slip of the extensor muscle (*t.e.m.*<sup>a</sup>) of the caudal spine, which is attached to the last three entapophyses (*enta.*<sup>12-14</sup>). In this muscle it anastomoses with branches from the post-cardiac nerves which supply the same muscle. The connection of the cardiac branch of this neuromere with the pericardial nerve (*p.n.*) is also irregular; sometimes this connection is entirely absent and some of its branches pass backwards in the epidermis to the posterior margin of the carapace. Whether these branches are prolongations of the pericardial nerve, or merely branches of the last cardiac nerve, is difficult to say.

(c) *Integumentary Branches.*—The lateral expansions of the carapace are very thin just outside of the bases of the appendages, but very thick at the outer margins. The integumentary branches, therefore, do not show a distinct division into haemal and neural branches except at their distal ends. Near the base of each appendage a small nerve is given off posteriorly to the epidermis. The main integumentary branches (Pl. VI,

Fig. 1) proceed diagonally backward and outward to the rim of the carapace. The fibers show a tendency to separate from each other as they approach the thicker margin and eventually break up into several divisions; one branch goes haemally, another neurally, and small branches go posteriorly and anteriorly, but the principal branch continues outward to one of the six large marginal spines (*a.s.<sup>9-14</sup>*), which are attached to the sides of the abdominal carapace. The branch from the first (*h.n.<sup>9</sup>*) branchial neuromere enters the first of these spines, where it breaks up into fine fibers. The first five spines are innervated by nerves from the corresponding five branchial neuromeres.

f. *Nerves from the Post-Branchial Neuromeres.* — As there are no appendages in the post-branchial metameres, and the neuromeres are indistinguishably fused, it is difficult to follow the metamerism in this region. In the typical neuromeres the neural nerves supply the appendages exclusively, and the haemal nerves the remainder of the metamere. In the post-branchial metameres there are no appendages. If we consider the neural nerves as absent, the post-branchial nerves fall into three similar pairs, which partake very strongly of the nature of the typical haemal nerves.

(1) *Nerves from the First Post-Branchial Neuromere.* — The first post-branchial nerve (Pls. VI, VIII, and IX, Figs. 1, 3-5, *h.n.<sup>14</sup>*) is very similar to the last haemal branchial nerve. It passes out from the fused, terminal, ganglionic mass posterior to the muscles of the last gill and anterior to the last haemo-neural muscle (*h.n.m.<sup>14</sup>*), and goes diagonally backward toward the margin of the carapace. Like the typical haemal nerve, it is divisible into an intestinal branch, a post-cardiac branch, and an integumentary branch.

(a) *Intestinal Branches.* — In this neuromere the intestinal branches (*i.n.<sup>14</sup>*) arise sometimes at some distance from the ganglionic mass, and the muscular and visceral branches may arise quite independently of each other (Pl. VIII, Fig. 4). The branch supplying the last haemo-neural muscle, which belongs to this metamere, has not been found.

(b) *Post-Cardiac Branch.* — The post-cardiac branch (*s.c.n.<sup>14</sup>*) arises at some distance from the proximal end of the nerve and

passes haemally just anterior to the last entapophysis (*enta.<sup>14</sup>*). A branch is given to the lateral sympathetic before the post-cardiac nerve separates from the main nerve. This is the posterior limit of the lateral sympathetic (Text-fig. 18). Near the entapophysis the post-cardiac nerve gives off branches to a slip of the extensor muscle (*t.e.m.<sup>a</sup>*) of the caudal spine, which is attached to the last three entapophyses. In this muscle the branches anastomose anteriorly with the corresponding branches from the last branchial neuromere, and posteriorly with a similar branch from the second post-branchial neuromere. The post-cardiac nerve terminates in the epidermis in the haemal median line posterior to the heart.

(c) *Integumentary Branches.*—The integumentary branch passes diagonally backward to the thickened rim of the carapace, where it gives off haemal and neural branches and some small nerves posteriorly and anteriorly, but the main branch supplies the last large spine (*a.s.<sup>14</sup>*) upon the edge of the carapace.

(2) *Nerves from Second Post-Branchial Neuromere.*—This pair of nerves (*h.n.<sup>15</sup>*), because of their larger area of distribution, are somewhat larger than the preceding haemal nerves. They arise from the terminal ganglionic mass just back of the first post-branchial nerve, and pass posteriorly and outward posterior to the last haemo-neural muscle (*h.n.m.<sup>14</sup>*). This nerve is also divisible into intestinal, post-cardiac, and integumentary branches.

(a) *Intestinal Branches.*—From the base of the nerve (*i.n.<sup>15</sup>*) a small fiber passes backward along the intestine to which it gives off several branches. It then continues backward nearly to the rectum, where it unites with a nerve (*i.n.<sup>16</sup>*) from the third post-branchial neuromere. No muscular branches have been found.

(b) *Post-Cardiac Branch.*—Posterior to the last haemo-neural muscle a large branch goes haemally between the slips of the flexors of the caudal spine. Near the haemal side of the body this branch divides into two; one going outward to the external slips of the extensors (*t.e.m.<sup>b</sup>*) of the caudal spine, and to the epidermis in the neighboring region; the other going toward



the median line to the internal slips of the extensors (*t.e.m.<sup>a</sup>*), and to the epidermis in the median line. Some of the branches go to the posterior margin of the carapace over the base of the caudal spine. Inside the extensor muscles the branches anastomose with those of the first post-cardiac nerve.

(c) *Integumentary Branches.*—Besides the integumentary portion of the post-cardiac, which innervates the epidermis upon the haemal side of the carapace, there is a large integumentary branch of the main nerve, which innervates the neural side of the carapace. After giving off the post-cardiac branch, the main nerve enters the flexor muscles of the caudal spine and gives off to these muscles branches which anastomose with similar branches from the third post-branchial nerve (*h.n.<sup>16</sup>*). The integumentary portion turns posteriorly and supplies the epidermis in the posterior angles of the abdominal carapace.

(3) *Nerves of the Third Post-Branchial Neuromere.*—The terminal ganglionic mass sends out a large pair of nerves (*h.n.<sup>16</sup>*), which pass posteriorly upon each side of the rectum to the telson. Each of these nerves divides, sometimes almost at the very base, into two branches. The first goes between the flexor (*t.f.m.*) and extensor (*t.e.m.<sup>b</sup>*) muscles of the telson and gives to the flexor (*t.f.m.*) branches which anastomose with the branches of the second post-branchial nerve. The distal or integumentary portion divides, sending one branch to the posterior angle of the carapace, and the other to the telson. This nerve also sends some fine branches to the epidermis in the posterior margin of the carapace neural to the base of the telson.

The second branch, which is much the larger of the two, breaks up in the telson into numerous branches. At the side of the rectum it gives off a branch (*i.n.<sup>16</sup>*) which supplies the anal muscles and the rectum and communicates with the intestinal nerve (*i.n.<sup>15</sup>*) of the second post-branchial neuromere. A branch of this nerve also goes to the epidermis at the base of the telson, posterior to the anus.

Milne-Edwards describes a small ganglion upon each side of the anus at the root of the nerve going to the rectum, but we have failed to find it. This ganglion is supposed to lie inside

the large blood vessel which accompanies the last haemal nerve into the telson. In alcoholic specimens white blood clots are often found in the forks of the arteries, and these might easily be mistaken for ganglia in a gross dissection. Such a clot is often found at the spot designated by Milne-Edwards, but microscopic examination reveals no ganglion cells.

There are no post-cardiac branches in the terminal neuromere.

### 3. SYMPATHETIC SYSTEMS.

#### a. *Lateral Sympathetic.*

Milne-Edwards in 1873 described the lateral sympathetic as a lateral longitudinal nerve parallel to the ventral cord. Referring to the abdominal haemal nerves he says: "Chacun d'eux envoie un filet qui se dirige en avant, et va se réunir, ou plutôt concourt à former un nerf latéral longitudinal. Celui-ci" (the lateral sympathetic nerve) "s'étend parallèlement à la chaîne ganglionnaire, un peu en dehors de la veine collectrice et entre les muscles abdominal-oblique et branchio-thoracique; il se prolonge en avant jusqu'au thorax, et en arrière il présente un petit renflement ganglionnaire; dans son parcours il fournit des filets aux muscles voisins. Ce nerf latéro-abdominal, dont l'existence n'a jusqu'à présent été signalée que chez les *Limulus*, rappelle par sa position le grand sympathique des animaux supérieurs; mais son rôle physiologique est tout à fait différent, puisque au lieu de se distribuer aux organes de la vie de nutrition, il se rend aux organes de la vie de relation."

I have found that the lateral sympathetic nerve (Text-figs. 9, 15, 16, and 18; Pls. VI, VIII, and IX, Figs. 1, 3, and 6, *l.s.n.*), which has already been partially described, lies nearly parallel to the ventral cord, and receives a branch from each of the haemal nerves, from the eighth to the fourteenth, inclusive. It is in close connection with the branchio-thoracic muscles (*b.t.m.*), and its anterior portion is formed of anastomosing branches within, and forming the nerve supply of, this bundle of muscles. It extends as far forward into the cephalothorax as do the branchio-thoracic muscles. The posterior portion consists



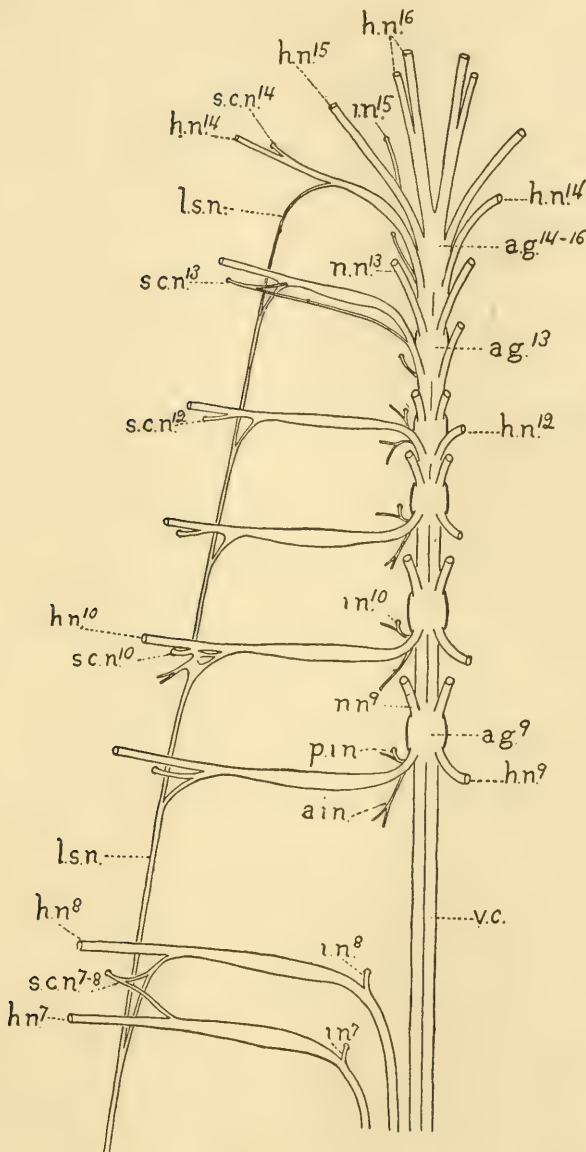


FIG. 18. — Diagram showing the lateral sympathetic nerve and its relations to the haemal and cardiac nerves and the ventral cord (seen from the neural side).

*a.g.*<sup>9-16</sup>, abdominal ganglia; *a.i.n.*, anterior branch of intestinal nerve of ninth neuromere; *h.n.*<sup>7-16</sup>, haemal nerves of the seventh to sixteenth neuromeres; *i.n.*<sup>7-15</sup>, intestinal nerves of the seventh to fifteenth neuromeres; *l.s.n.*, lateral sympathetic nerve; *n.n.*<sup>9-13</sup>, neural nerves of the ninth to thirteenth neuromeres; *p.i.n.*, posterior branch of intestinal nerve of ninth neuromere; *s.c.n.*<sup>7-13</sup>, segmental cardiac nerves of the seventh to the fourteenth neuromeres; *v.c.*, ventral cord.

of a single nerve trunk lying, for the most part, neural to, or outside of, the tendinous stigmata (*t.s.*<sup>8-13</sup>).

The branchio-thoracic muscles do not extend back as far as the lateral sympathetic nerve goes, but their posterior portions are replaced by the tendinous stigmata, which are segmental invaginations of the chitin furnishing attachment for the branchio-thoracic muscles. The tendinous stigmata, six in number, occur just posterior to each of the abdominal appendages; *i.e.*, from the eighth to the thirteenth metameres, inclusive. The branchio-thoracic muscles, which are attached to these stigmata, belong, then, in the eighth to thirteenth metameres, and should receive their nerve supply from the eighth to thirteenth neuromeres. This is actually the case; the lateral sympathetic nerve, or more properly the branchio-thoracic nerve, receives a recurrent branch from each of the haemal nerves from the eighth to thirteenth (*h.n.*<sup>8-13</sup>); but it also receives one, a very fine one, from the fourteenth haemal nerve. This would indicate that there is a branchio-thoracic muscle belonging to the fourteenth metamere, which is very probable, inasmuch as we have a pair of entapophyses, a pair of haemoneural muscles, and a pair of typical haemal nerves in this metamere, but no appendage or neural nerves.

The recurrent branches of the lateral sympathetic arise from the haemal nerves near the segmental cardiac nerves (*s.c.n.*<sup>8-14</sup>), or from the roots of the cardiac nerves themselves. The exact mode of origin varies considerably in different individuals; Fig. 18 (text) shows the relation of the lateral sympathetic to the haemal nerves in one specimen.

The cardiac nerves (*s.c.n.*<sup>7 and 8</sup>) from the seventh and eighth haemal nerves fuse together, and the root of the eighth cardiac nerve gives off a branch to the sympathetic.

The root of the ninth cardiac nerve (*s.c.n.*<sup>9</sup>) also sends a branch to the sympathetic.

In the tenth neuromere the roots of the cardiac (*s.c.n.*<sup>10</sup>) and recurrent branches are flattened out, and some small nerves are given off to the inter-entapophysial muscles. The origin of the recurrent branch is, however, essentially the same as in the preceding neuromere, except that the cardiac nerve does not

separate entirely from the haemal nerve before the recurrent branch is given off.

In the eleventh and twelfth neuromeres the recurrent branches are given off from the haemal nerves (*h.n.<sup>11</sup>* and *12*) before the cardiac branches (*s.c.n.<sup>11</sup>* and *12*) separate from them.

A portion of the cardiac branch (*s.c.n.<sup>14</sup>*) of the fourteenth neuromere separates from the haemal nerve (*h.n.<sup>14</sup>*) near the ventral cord and, opposite the lateral sympathetic, receives another fiber which separates from the haemal nerve close to the origin of the recurrent sympathetic branch.

The last recurrent sympathetic branch arises from the fourteenth haemal, or first post-branchial nerve (*h.n.<sup>14</sup>*). The post-cardiac (*s.c.n.<sup>14</sup>*) separates from the haemal nerve some distance beyond the origin of the sympathetic branch.

Under favorable conditions small recurrent fibers may be seen in the angles between the roots of the sympathetic branches and the outer, or integumentary portions of the haemal nerves.

The ganglionic enlargements referred to by Milne-Edwards could not be found. He probably referred to the flattened portions which sometimes occur at the junctions of the lateral sympathetic with the haemal nerves. These flattenings are likely to occur wherever a nerve passes between two muscles or other parts in close contact with each other; they do not contain any ganglion cells.

#### b. *Nerves of the Heart.*

The only mention we have of the cardiac nerves of *Limulus* is found in "Anatomie des *Limulus*," by Milne-Edwards. "Sur les côtés de l'estomac se trouvent aussi deux filets délicats et ténus qui se rendent à un nerf volumineux situé sur la ligne médiane du cœur et dans toute la longueur de cet organ. Ce nerf cardiaque, qui s'amincit beaucoup vers les extrémités du vaisseau dorsal, est au contraire très-large vers la partie moyenne de celui-ci; effectivement, il présente un certain nombre de renflements situés au niveau de chaque paire d'ouvertures vasculaires, et de ces points partent des filets qui se dirigent à droite et à gauche sur les parois adjacentes."

The plexus upon the heart, and its connections with the central nervous system through the segmental cardiac nerves have never been described. The "deux filets délicats et ténus" which Milne-Edwards describes, but does not represent in his figures, we have not been able to find.

(1) *The Cardiac Plexus.* — In a cross-section of the heart (Text-figs. 12 and 14; Pl. IX, Figs. 6 and 7) three large nerves (*l.c.n.* and *m.c.n.*) are seen in the three angles. They lie upon the outside of the heart between the longitudinal strands of connective tissue. In Figs. 5, 8, 9, and 10 of the plates these nerves are seen to better advantage.

A median ganglionated nerve (*m.c.n.*) traverses the heart longitudinally upon the haemal side. Along the middle of its course it is quite large, but dwindles down at the ends. Under a low magnifying power (Pls. IX and X, Figs. 8 and 9) it appears as a large bundle of intertwining fibers intermingled with masses of ganglion cells. Still higher magnification shows that many of these ganglion cells are bipolar (*g.c.*, Pl. X, Fig. 10).

The lateral nerves (*l.c.n.*) of the heart are not ganglionated. They traverse the sides of the heart just above the lateral angles and communicate with the median nerve by an elaborate plexus, which is richest upon the haemal sides of the heart. The neural side of the heart seems to have very few nerves.

The main branches of the cardiac plexus (Pl. IX, Fig. 8) arise from the median nerve uniformly in pairs opposite the ostia, but the connections with the lateral nerves seem to be entirely irregular. Some of the branches of the plexus approach very closely the origins of the lateral arteries, but no nerves have been observed running out onto them.

(2) *Segmental Cardiac Nerves.* — The segmental cardiac nerves (Text-figs. 8, 9, 12, 14, 15, 16, and 18, Pls. VI–IX, 1–3, 5, and 6, *s.c.n.*<sup>6-15</sup>) have been partially described in the foregoing pages. Those of the five branchial neuromeres (*s.c.n.*<sup>9-13</sup>) are most typical, and they will be considered first. They arise from the haemal nerves (*h.n.*<sup>9-13</sup>), in close connection with the recurrent branches of the lateral sympathetic, opposite the branchio-thoracic muscles, and pass outside of these muscles to the haemal side of the body, just anterior to the entapophyses















(*enta.*<sup>9-13</sup>). They then turn toward the median line in the epidermis haemal to the heart, where they dip downward and communicate with the median nerve (*m.c.n.*) of the heart (Pl. IX, Figs. 5 and 6, and Text-fig. 8) opposite the last five pairs of ostia (*os.*<sup>9-13</sup>). The connections with the median nerve of the heart have been actually found only for the cardiac nerves (*s.c.n.*<sup>9-13</sup>) of the five branchial neuromeres, but similar connections probably exist in other neuromeres.

Besides the branch communicating with the median cardiac nerve, the segmental cardiac gives off numerous branches to the epidermis haemal to the heart, and also an important branch which goes anteriorly and unites with the pericardial nerve (*p.n.*), a longitudinal nerve trunk running parallel to the heart inside the pericardium.

The cardiac branches (*s.c.n.*<sup>7 and 8</sup>) of the seventh and eighth neuromeres fuse together and form a large nerve, which passes haemally outside of the branchio-thoracic muscles and anterior to the large entapophysis (*enta.*<sup>7 and 8</sup>). The root from the eighth neuromere gives a branch to the lateral sympathetic. Upon the haemal side of the body this cardiac nerve divides into a number of branches. A large one enters the inter-tergal muscle and breaks up into anastomosing branches which supply that muscle. Some small branches pass into the epidermis haemal to this muscle, and approach the median line. Although these branches could be traced in the epidermis to points just above the three anterior pairs of ostia (*os.*<sup>6-8</sup>), no connections with the median nerve of the heart could be made out. In this region the connective-tissue strands which support the heart upon the haemal side are very numerous, and it is difficult to trace nerve fibers among them.

The most important branch of this cardiac nerve, the pericardial nerve (*p.n.*), turns posteriorly in the areolar tissue which lies above the pericardial sinus. It first gives off a small branch to the lateral inter-tergal muscle, and then, continuing posteriorly, gives a branch to each of the cardiac nerves (*s.c.n.*<sup>9-13</sup>) of the branchial neuromeres. These branches pass from the outer side of the pericardial nerve toward the proximal ends of the cardiac nerves. The posterior extremity of the

pericardial nerve is lost in a plexus of nerves, some of the branches of which extend in the epidermis to the posterior margin of the abdomen.

The cardiac nerve (*s.c.n.*<sup>13</sup>) of the last branchial neuromere differs from the others in that it gives a branch to a slip of the extensor muscle of the telson, which is inserted upon the last three entapophyses. Similar branches from the first two post-cardiac nerves go to the same muscle, anastomose with this nerve and with each other, and send a branch in the epidermis to the posterior end of the abdomen.

The two post-cardiac nerves (*s.c.n.*<sup>14 and 15</sup>) also send branches to the epidermis near the median line. As the heart does not extend back of the thirteenth metamere, the post-cardiac nerves have no connection with this organ.

In the sixth thoracic neuromere a large cardiac branch (*s.c.n.*<sup>6</sup>) is given off from the sixth haemal nerve (*h.n.*<sup>6</sup>). This does not communicate with the lateral sympathetic, but passes haemally and anteriorly outside of the branchio-thoracic muscles, and enters the large inter-tergal muscles haemal to the heart. Here its branches anastomose with those of the fused seventh and eighth cardiac nerves (*s.c.n.*<sup>7 and 8</sup>). Some of the finer branches innervate the epidermis also, and possibly communicate with the median nerve of the heart, although this connection has not been observed.

A small nerve, which could not be traced out, was found arising from the fifth haemal nerve (*h.n.*<sup>5</sup>) at a point corresponding to the origins of the cardiac nerves of other neuromeres.

It is a curious fact that, leaving out the post-cardiac nerves, there are as many segmental cardiac nerves as there are ostia in the heart. The cardiac nerves (*s.c.n.*<sup>9-13</sup>) from the five branchial neuromeres enter the heart opposite the five posterior pairs of ostia (*os.*<sup>9-13</sup>). This leaves three segmental cardiac nerves (*s.c.n.*<sup>6-8</sup>) corresponding to the three anterior pairs of ostia (*os.*<sup>6-8</sup>). If these have any connections with the median nerve of the heart, the connection will probably be found opposite the three anterior pairs of ostia, inasmuch as the connections of the other five cardiac nerves with the median cardiac



nerve have been found opposite the five posterior pairs of ostia. The stump of the cardiac nerve found in the fifth neuromere would then correspond to the rudimentary ostia (*r.os.*).

c. *Nerves of the Alimentary Tract.*

(1) *The Rostral Nerves.* — Three rostral nerves, a median and two lateral ones (Pls. VI–VIII, and X, Figs. 1–3, 11, and 12, *l.a.n.*), arise from the anterior commissure and innervate the rostrum, or upper lip.

(2) *Stomodaeal Nerves.* — A pair of stomodaeal nerves (Pls. VIII and X, Figs. 3, 11, and 12, *st.n.*) arise from large ganglia on the inner side of the oesophageal collar and innervate the oesophagus, proventriculus, and pyloric valve. These have already been fully described under the *Nerves from the Mid-Brain.*

A small ganglion upon the sides of the proventriculus, the existence of which is doubtful, has been described by Milne-Edwards. “Au point où cet organe se replie brusquement pour se porter en arrière, se trouve un très-petit ganglion aplati, logé, comme le nerf dans l’artère, près de l’anastomose de cette dernière avec la branche gastrique émanée de la convexité de la crosse aortique.” The point mentioned is a place within the aortic arch from which several nerves diverge to supply the proventriculus, and where white blood clots are extremely liable to lodge. In alcoholic specimens these clots take on the appearance of ganglia.

“De ce ganglion partent en avant des filets qui se distribuent aux parois très-muscleuses de l’estomac, et en arrière deux rameaux dont l’un se rend à la portion pylorique de ce viscère, et l’autre gagne l’intestin. Ces parties sont très difficiles à distinguer, car elles sont extrêmement grêles, et pour les dégager des artères où elles sont logées, il faut procéder avec un très grand soin.” I have found branches running onto the anterior end of the intestine, but could not trace them beyond the pyloric valve even with a methylen blue stain. Although good stains of the nerves of the proventriculus and the anterior end of the intestine were easily obtained by this method, every

attempt to demonstrate a nerve plexus on the intestine has failed.<sup>1</sup>

According to Milne-Edwards "Sur les côtes de l'estomac se trouvent aussi deux filets délicats et ténus qui se rendent à un nerf volumineux situé sur la ligne médiane du cœur et dans toute la longueur de cet organe." All attempts to find these cardiac branches of the stomodaeal nerves have been unsuccessful.

(3) *Intestinal Nerves*.—Milne-Edwards has described, in addition to the stomodaeal nerves, a pair innervating the posterior portion of the alimentary canal. "L'autre, destiné à l'épine caudale, passe au-dessous du muscle abaisseur de l'anús et a point où l'artère qui le contient s'anastomose avec l'artère anastomotique, fournit trois ou quatre filets grêles qui remontent sur les parois de l'intestin et se rendent à un petit ganglion rectal situé un peu en avant du sphincter de l'anús, au-dessus du faisceau musculaire abaisseur de celui-ci. Ce ganglion, un peu allongé d'avant en arrière, est logé dans la dilatation artérielle qui existe sur ce point, et envoie des filets nombreux en avant, en dessus et en arrière. Ces filets s'enfoncent dans les parois intestinales. L'existence de ce petit centre ganglionnaire est très-curieuse et indique un système sympathique rectal qui n'existe pas, ou du moins qui n'a pas été observé chez les autres Arthropodes; il est d'ailleurs très-difficile à isoler des parois artérielles qui l'engainent."

As in the case of the ganglion upon the side of the proventriculus, the existence of the rectal ganglion is doubtful. Clots of blood have been observed at this point in dissecting, and careful examination of serial sections through this region has revealed no ganglion cells. A mass of matter with many small nuclei was found, but this had exactly the appearance of undoubted blood clots in other portions of the same artery.

Hitherto the segmental intestinal nerves have not been described. These occur in all the neuromeres from the sixth to the sixteenth, and possibly in the second neuromere. Their

<sup>1</sup> More recent trials indicate the presence of an elaborate nerve plexus provided with minute ganglion cells surrounding the circular muscles of the intestine.

arrangement is most typical in the abdominal region, and it will be well to take up one of these neuromeres first.

In the first gill neuromere (Text-fig. 8; Pls. VIII and IX, Figs. 3, 4, and 6) two small nerves arise very close together from the haemal side of the haemal nerve (*h.n.*<sup>9</sup>) close to the abdominal ganglion. The anterior one (*a.i.n.*) enters the adjacent mass of longitudinal abdominal muscles and communicates with the plexus supplying these muscles. The posterior one (*p.i.n.*) divides into two branches, one going to the haemo-neural muscle (*h.n.m.*<sup>9</sup>) and the other to the intestine. The latter has been observed to communicate with the corresponding nerves of other neuromeres by a plexus lying in the tissue immediately surrounding the intestine. Only glimpses of this plexus have been obtained here and there. The nerves are very fine and not easily made out without a microscope. In one or two instances, while working with methylen blue, a fine plexus was seen in the tissues haemal to the intestine, near the posterior end.

In the tenth, eleventh, and twelfth neuromeres the roots of the two intestinal nerves arise a little farther apart. In each case the anterior nerve joins the plexus in the longitudinal muscles. The posterior nerve gives a branch to the haemo-neural muscles, and one to the intestine, and also communicates sometimes with the plexi in the longitudinal abdominal muscles, and in the tissues surrounding the intestine.

In the neuromeres posterior to the twelfth the intestinal nerves are very irregular in their origin, sometimes arising from the haemal nerve at some distance from the ganglion, and entering the intestine by several branches. In the specimen represented in Fig. 4 of the plates the posterior branch of the intestinal nerve (*i.n.*<sup>12</sup>) of the twelfth neuromere traverses the twelfth haemo-neural muscle (*h.n.m.*<sup>12</sup>) before entering the intestine. In this figure the branches going to the intestine are represented as cut off.

The next intestinal nerve (*i.n.*<sup>13</sup>) has no muscular branch.

The fourteenth (*i.n.*<sup>14</sup>) upon the right side arises near the abdominal ganglion and proceeds posteriorly a long distance, and finally enters the intestine by three branches. Upon the left side the fourteenth intestinal nerve (*i.n.*<sup>14</sup>) is

represented by three branches arising from the haemal nerve (*h.n.<sup>14</sup>*) at some distance from the ganglion. One of these enters the longitudinal abdominal muscles, the second anastomoses with the twelfth intestinal nerve (*i.n.<sup>12</sup>*), and the third enters the intestine.

The fifteenth intestinal nerve (*i.n.<sup>15</sup>*) arises near the origin of the fifteenth haemal nerve (*h.n.<sup>15</sup>*) and passes posteriorly along the surface of the intestine, giving off several branches to the intestine, and then anastomoses with the sixteenth intestinal nerve (*i.n.<sup>16</sup>*).

The sixteenth intestinal nerve (*i.n.<sup>16</sup>*) arises from the caudal branch of the sixteenth haemal nerve (*h.n.<sup>16</sup>*) about midway between the terminal abdominal ganglion and the anus, and passes posteriorly a short distance to the side of the rectum, or proctodaeum, where it divides into several branches. It was at this point that Milne-Edwards found the "ganglion rectal." One branch goes anteriorly to anastomose with the fifteenth intestinal nerve (*i.n.<sup>15</sup>*), a second one goes posteriorly to the *levator ani* (*l.a.*) and to the rectum, and the third goes to the epidermis upon the haemal side of the base of the telson.

In one methylen blue preparation the sixteenth pair of intestinal nerves were joined together by a cross-branch upon the neural side of the rectum. In some of the anterior neuromeres also a similar cross-branch has been found uniting the intestinal nerves of the right and left sides of the body. This seems to suggest that there is an elaborate network of nerves in the tissues surrounding the intestine. As these nerves are very delicate, it is difficult to trace them by dissection alone, and many of them with their connections are necessarily missed.

The intestinal nerves (*i.n.<sup>6-8</sup>*) of the sixth, seventh, and eighth neuromeres are so complicated in their relations that it is impossible to unravel them. The sixth (*i.n.<sup>6</sup>*) and seventh (*i.n.<sup>7</sup>*) pass through foramina (*f.<sup>6</sup>* and *f.<sup>7</sup>*) in the endocranium and lose themselves in the anterior portion of the mass of longitudinal abdominal muscles. The eighth (*i.n.<sup>8</sup>*) goes directly into these muscles near the posterior edge of the endocranium. Sometimes each one of these nerves arises from the haemal nerve



by two separate roots, and the seventh has been observed to pass through the endocranium by two foramina.

They commingle in a rich plexus supplying the longitudinal abdominal muscles. From this plexus numerous branches emerge and, after ramifying and anastomosing through the tissues outside of the intestine, enter its walls, some of them extending far forward toward the anterior end.

The intestinal nerve (*i.n.*<sup>2</sup>) arising from the second haemal nerve (Text-fig. 8; Pls. VIII and X, Figs. 3, 11, and 12) passes median to the anterior cornua of the endocranium and supplies the tergo-proplastral muscles (*t.p.m.*<sup>a and b</sup>), but no branch has been observed going to the intestine. As it has an origin similar to that of the other intestinal nerves, and supplies similar muscles, it has been included in the same category.

#### SUMMARY.

1. The nervous system of *Limulus* is made up of sixteen neuromeres exclusive of the fore-brain.

2. Each neuromere consists of a pair of ganglia united by several cross-commissures, a pair of neural and a pair of haemal nerves. In the first or cheliceral neuromere we have in addition a pair of stomodaeal and three rostral nerves. In the three post-branchial neuromeres the appendages and, consequently, the neural nerves are absent.

3. Each neuromere, as a rule, innervates one metamere; the neural nerves and their branches supply the appendages and the haemal nerves supply the remainder or body portion of the metamere, including the epidermis and internal organs.

But there are many cases in which nerves extend through several metameres either as single nerves or united with others to form longitudinal connectives. For example, the pericardial nerve springs from the fused seventh and eighth neuromeres and communicates with the corresponding nerves of the next five posterior neuromeres. The nerves which supply longitudinal muscles extending through several metameres and having muscular slips attached in each of the metameres are united into longitudinal anastomosing plexuses. The lateral sympa-

thetic, the plexus in the flexors and in the extensors of the telson, and the anterior branches of the segmental cardiac nerves supplying the median inter-tergal muscles, are similar examples. In fact, wherever muscles of different metameres fuse together, we find the nerves supplying them united into longitudinal connectives. The first haemal nerve or lateral nerve is an exceptional case in that it extends through nearly all metameres of the body, but does not communicate with any of the other haemal nerves except the second.

4. The brain may be divided into four regions: (1) the fore-brain, or cerebral lobes, which is probably formed of three neuromeres, an olfactory, a median eye, and a lateral eye neuromere; (2) the mid-brain, formed of the cheliceral neuromere; (3) the hind-brain, formed of five thoracic neuromeres, those from the second to the sixth; and (4) the accessory brain, formed of two neuromeres, the chilial, or seventh neuromere, and the opercular, or eighth neuromere, both of which were originally abdominal neuromeres.

5. The neuromeres of the accessory brain region, the chilial and opercular neuromeres, are more completely united than those in front of or behind them, and some of their nerves wander into other metameres than their own. This fact led Dr. Patten to call the accessory brain the "vagus region."

The cardiac nerves of these two neuromeres are completely fused and on the haemal side of the body form the pericardial nerve, which extends into the five branchial neuromeres.

6. In the typical cranial neuromere the neural nerve divides into three groups of branches: (1) the mandibular branches; (2) the ento-coxal branches; and (3) the pedal branches. The haemal nerve also divides into three branches: (1) the intestinal branch; (2) the cardiac branch; and (3) the integumentary branch.

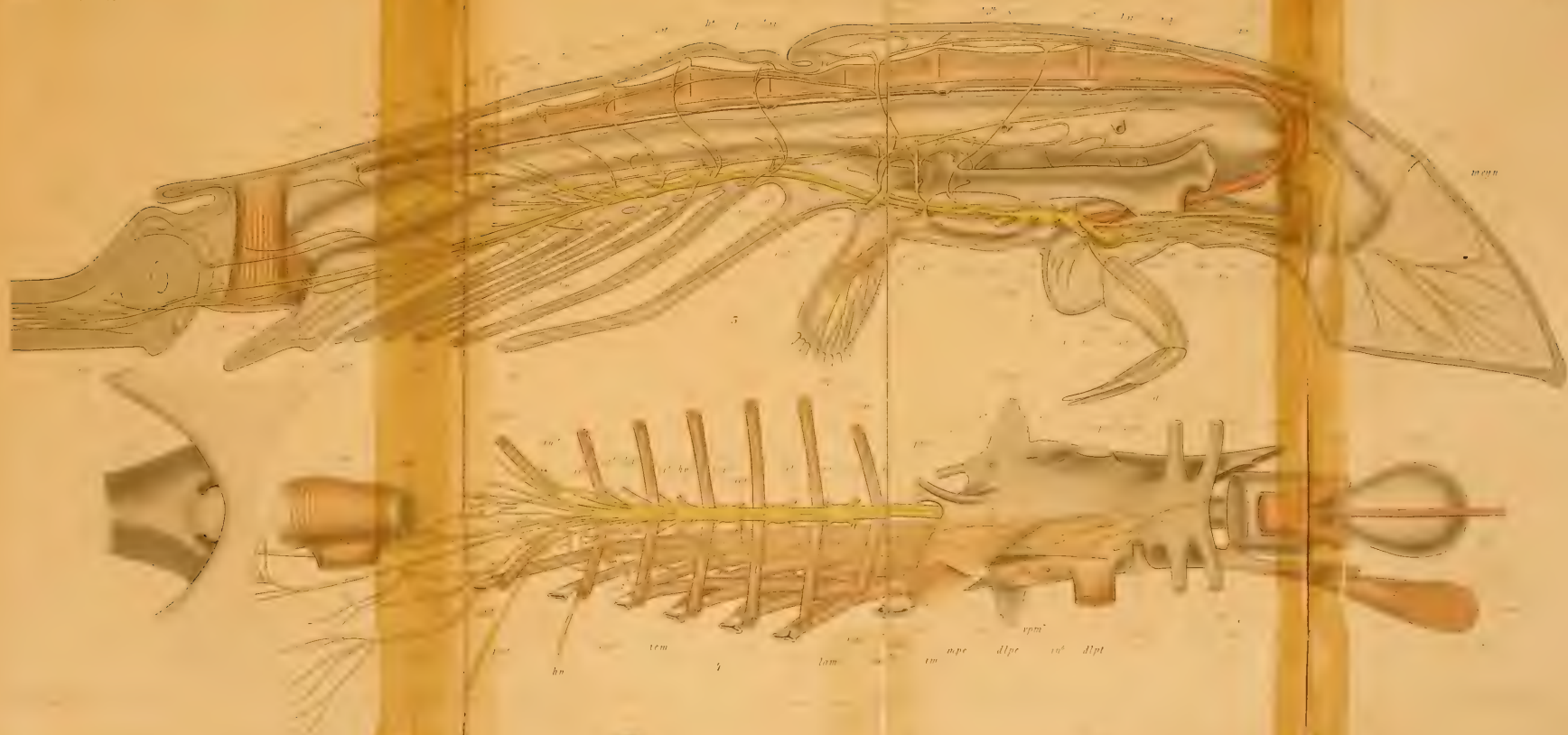
The ventral cord consists of five branchial neuromeres (those from the ninth to the thirteenth) and three post-branchial neuromeres (the fourteenth, fifteenth, and sixteenth).

In the typical abdominal neuromere the neural nerve arises from the posterior side of the ganglion and divides into three branches, one to the anterior and one to the posterior side











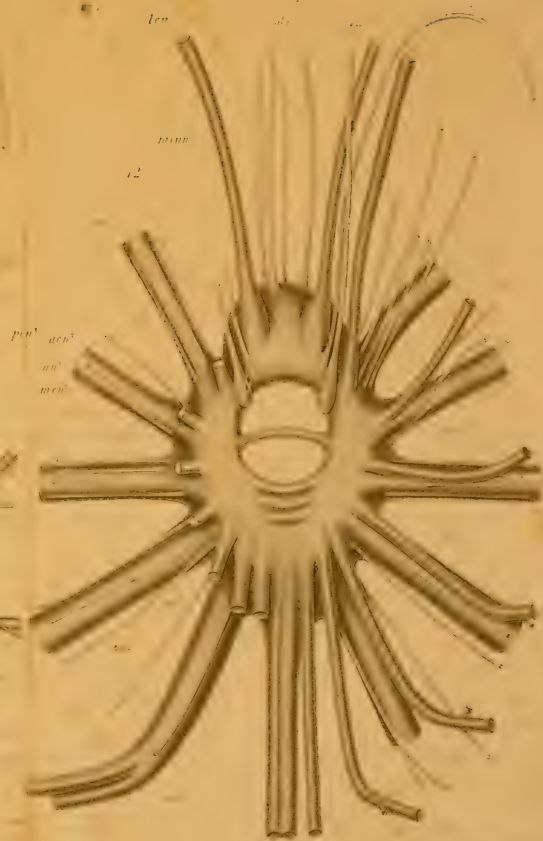














# THE MATURATION AND FERTILIZATION OF THE EGG OF LIMAX AGRESTIS (LINNÉ).

ESTHER FUSSELL BYRNES.

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## INTRODUCTION.

THE study of the maturation and fertilization of the egg of *Limax agrestis* was carried on in the Biological Laboratory of Bryn Mawr College, under the direction of Prof. T. H. Morgan. I gladly take this opportunity of thanking Professor Morgan for his kind interest and helpful guidance during the course of the work.

The development of the egg of *Limax agrestis* was first studied by Warneck in 1850; and in 1881 Mark published his

epoch-making classic on the maturation and fertilization of the egg of *Limax campestris*. The studies of Warneck on the living eggs of *Limax agrestis*, and of Mark on *Limax campestris*, leave little to be added to the descriptions that have already been given of the phenomena exhibited by the living eggs during the early stages of development. When, however, the eggs are preserved and sectioned, and then stained in haematoxylin after iron-alum, they show details of structure that cannot be seen either in the living egg or in preserved eggs studied in optical sections.

It will be convenient in describing the maturation stages to distinguish between the aster as a whole and the center of the aster, which undergoes a series of changes that are apparently independent of modifications of the astral rays. I shall, therefore, follow the terminology that Wilson has adopted in his studies on the sea-urchin's egg, and use the term "astrosphaere" to designate the astral rays as well as the center of the aster. For the center of the aster alone I shall reserve the term "centrosphere." This terminology is employed only for convenience in description, and has no significance based on the recognition of a fundamental distinction between the two parts of the astrosphaere.

## I. MATURATION OF THE EGG.

### 1. *The Archiamphiaster.*

The youngest eggs of *Limax campestris* that Mark studied were those that had just been deposited. In these eggs a large amphiaster, the "archiamphiaster" of Whitman and of Mark, occupied the center of the egg. The eggs of *Limax agrestis* agree very closely with those of *Limax campestris* in forming the archiamphiaster before the eggs are laid. Sections of eggs that have just been laid show that in the archiamphiaster stage the centers of the asters no longer appear structureless, as in the living egg, but are composed of distinct concentric rings or zones, which react toward haematoxylin and other staining reagents very differently from the rest of the cell. During



of the proximal portion of the appendage, and one to the distal portion; the haemal nerve arises from the anterior side of the abdominal ganglion and divides into three branches: (1) the intestinal branch; (2) the cardiac branch; and (3) the integumentary branch.

7. The rostrum, oesophagus, and proventriculus are innervated by the rostral and stomodaeal nerves, which arise respectively from the pre-oral commissure and from the ganglia on the median sides of the first thoracic neuromere.

The intestine and rectum are innervated by the intestinal nerves, which arise from all the neuromeres from the sixth to the sixteenth (possibly from the second also).

The intestinal branches of the sixth to the sixteenth neuromeres anastomose with each other and form plexi or longitudinal connectives parallel to the ventral cord.

8. The heart extends through all the metameres from the sixth to the thirteenth and is probably innervated by cardiac nerves from each of the neuromeres from the sixth to the thirteenth, although actual connections have been made out only in the five branchial neuromeres, *viz.*, the ninth to the thirteenth.

The cardiac branches of the vagus and abdominal neuromeres are joined to each other by longitudinal connectives or sympathetic branches, *viz.*, the lateral sympathetic and the pericardial nerves.

9. The muscles of each metamere are innervated from the corresponding neuromere. If the muscles of several metameres become fused with one another, the nerves of the different neuromeres anastomose with each other to form plexi.

10. The chilaria were regarded by Owen and Lankester as detached inner portions of the sixth pair of mandibles. This is without doubt an error, since in the embryo there is a separate neuromere for the chilaria, and in the adult, though the ganglia are fused with those of the sixth and eighth neuromeres, there is still a pair of distinct neural (*n.n.*<sup>7</sup>) and haemal nerves (*h.n.*<sup>7</sup>) belonging to the chilarial neuromere. The appendages also have a complicated set of muscles of their own, both plastrocoxals and tergo-coxals.

Moreover, the chilaria have certain characters which place

them together with the operculum, among the abdominal appendages. For example: (1) a bar of capsuliginous cartilage (*b.c.7*) acts as an internal support for the appendage, and this bar is identical in structure with the branchial bars found in the operculum and gills. The fact that this capsuliginous cartilage is found only in the branchial bars of the chilaria, operculum, and gills renders the resemblance the more striking. (2) The roof of the occipital ring (*oc.r.*) is very similar to the abdominal endochondrites (*a.e.<sup>8-13</sup>*). (3) The chilial muscles (*7<sup>c</sup>*) arising from the neural side of the occipital ring and inserted upon the insides of the chilaria may be compared to the internal branchial muscles (*i.b.m.<sup>9-13</sup>*); and the long tergo-coxal muscle (*7<sup>f</sup>*) of the chilaria resembles the external branchial muscles (*e.b.m.<sup>8-13</sup>*). (4) Its neural and haemal nerves are more like those in the abdomen than those of the thorax.

If we regard the chilial and opercular neuromeres as abdominal rather than cranial, and consider that the anterior parts of the intestine and probably of the heart are innervated from the sixth, seventh, and eighth neuromeres, then the intestine and the heart must be mainly abdominal organs, indicating a greater forward movement of the organs in the haemal than on the neural side of the body.

11. The innervation of the liver, nephridia, and generative organs has not been made out.

WM. A. REDENBAUGH.











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## EXPLANATION OF PLATES.

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In the colored plates nerves are represented in yellow, blood vessels in carmine, cartilage in blue, and muscles in brick red.

All exponential figures indicate the metamere to which the organ referred to belongs.

<i>a.</i>	anus.	<i>h.d.a</i> and <i>b</i>	hepatic ducts.
<i>a.c.</i>	anterior commissure of brain.	<i>h.n.</i> <sup>1-16</sup>	haemal nerves.
<i>a.cor.</i>	anterior cornu of endocranium.	<i>h.n.m.</i> <sup>8-14</sup>	haemo-neural muscles.
<i>a.e.</i> <sup>8-13</sup>	abdominal endochondrites.	<i>h.pr.</i>	haemal processes of endocranium.
<i>a.e.n.</i>	anterior ento-coxal nerve.	<i>ht.</i>	heart.
<i>a.i.n.</i>	anterior intestinal branch.	<i>i.b.m.</i>	internal branchial muscle.
<i>al.m.</i> <sup>5-13</sup>	alary muscles of heart.	<i>i.e.m.</i>	inter-entapophysial muscles.
<i>a.m.f.</i>	anastomosing muscle fibers of heart.	<i>i.m.</i>	inter-tergal muscle (entapophysial slip).
<i>ao.a.</i>	aortic arch.	<i>i.n.</i> <sup>2-16</sup>	intestinal nerves.
<i>ap.</i> <sup>1-13</sup>	appendages.	<i>int.</i>	intestine.
<i>a.s.</i> <sup>9-14</sup>	abdominal spines upon edge of carapace.	<i>la.</i>	labrum or rostrum.
<i>a.v.</i>	anterior valve of heart.	<i>la.</i>	levator ani (muscle).
<i>b.</i>	base of muscle fiber of heart.	<i>l.a.m.</i>	longitudinal abdominal muscles.
<i>b.c.</i> <sup>7-13</sup>	branchial cartilages.	<i>la.n.</i>	labral, or rostral, nerves.
<i>b.c.c.</i> <sup>8-13</sup>	branchio-cardiac canals.	<i>lar.</i> <sup>5-9</sup>	lateral arteries.
<i>b.mem.</i>	basement membrane of heart.	<i>l.c.</i>	lateral cornua of endocranium.
<i>b.t.m.a</i> and <i>b</i>	branchio-thoracic muscles.	<i>l.c.n.</i>	lateral cardiac nerves.
<i>c.ar.</i>	collateral artery.	<i>l.c.s.</i>	longitudinal connective-tissue strands of heart.
<i>d.l.p.e.</i>	dorso-lateral plastro-entapophysial muscle.	<i>l.e.</i>	lateral eye.
<i>d.l.p.t.</i>	dorso-lateral plastro-tergal muscle.	<i>l.e.n.</i>	lateral eye nerve.
<i>e.b.m.</i> <sup>8-13</sup>	external branchial muscles.	<i>l.n.</i>	lateral nerve.
<i>endo.</i>	endocranium.	<i>l.ol.n.</i>	left olfactory nerve.
<i>ent.</i> <sup>2-6</sup>	entocoxites.	<i>l.p.pr.</i>	latero-posterior process of endocranium.
<i>enta.</i> <sup>7-14</sup>	entapophyses.	<i>l.s.n.</i>	lateral sympathetic nerve.
<i>f.</i> <sup>6</sup> and <i>7</i>	foramina of endocranium.	<i>m.</i>	mouth.
<i>f.ar.</i>	frontal artery.	<i>m.c.n.</i>	median cardiac nerve.
<i>f.br.</i>	fore-brain.	<i>m.e.</i>	median eye.
<i>g.c.</i>	ganglion cells in median nerve of heart.	<i>m.e.n.</i>	median ento-coxal nerve.
<i>h.</i>	haemal branch of integumentary nerve.	<i>mes.</i>	meso-metasoma or abdomen.
		<i>m.ey.n.</i>	median eye nerve.
		<i>m.n.</i> <sup>2-6</sup>	mandibular nerves.
		<i>m.ol.n.</i>	median olfactory nerve.

<i>m.p.e.</i>	meso-plastral-entapophy-	<i>r.ol.n.</i>	right olfactory nerve.
	sial muscle.	<i>r.os.</i>	rudimentary ostia.
<i>n.<sup>2-5</sup></i>	nephridia.	<i>s.a.</i>	sphincter ani (muscle).
<i>n.n.<sup>1-13</sup></i>	neural nerves.	<i>s.a.ar.</i>	superior abdominal artery.
<i>n.o.</i>	nephridial opening.	<i>s.c.n.</i>	segmental cardiac nerve.
<i>o.a.</i>	occludor ani (muscle).	<i>st.n.</i>	stomodaeal nerve.
<i>oc.r.</i>	occipital ring.	<i>s.v.<sup>6-9</sup></i>	semilunar valves.
<i>oe.</i>	oesophagus.	<i>tel.</i>	telson.
<i>ol.or.</i>	olfactory organ.	<i>t.e.m.<sup>a</sup> and b</i>	extensor muscles of tel-
<i>os.<sup>6-13</sup></i>	ostia of heart.		son.
<i>p.</i>	pericardium.	<i>t.f.m.</i>	flexor muscles of telson.
<i>p.e.n.</i>	posterior ento-coxal nerve.	<i>t.p.m.<sup>a-c</sup></i>	tergo-proplastral muscles.
<i>p.i.n.</i>	posterior intestinal	<i>t.s.<sup>8-13</sup></i>	tendinous stigmata.
	branch.	<i>v.ar.</i>	ventral artery.
<i>p.n.</i>	pericardial nerve.	<i>v.c.</i>	ventral cord.
<i>p.o.c.<sup>2-5</sup></i>	post-oral commissures.	<i>v.c.s.</i>	venous collecting sinus.
<i>p.pr.</i>	posterior process of endo-	<i>v.p.m.<sup>6-13</sup></i>	veno-pericardiac muscles.
	cranium.	<i>x.</i>	root of supposed cardiac
<i>proc.</i>	proctodaeum.		nerve of fifth neuro-
<i>pros.</i>	prosoma or cephalotho-		mere.
	rax.	<i>x.</i>	four tergo-coxal muscles
<i>prov.</i>	proventriculus.		of chelicerae.
<i>p.s.</i>	pericardial sinus.	<i>7<sup>a</sup> and 7<sup>c</sup></i>	muscles of chilaria.



## EXPLANATION OF PLATE VI.

FIG. 1. A view of the entire nervous system of *Limulus* from the neural side. (About natural size.)

The carapace is represented as transparent, and all tissues which would obscure the nerves and internal organs are left out of the drawing. The appendages have been removed, but the outlines of the entocoxites (*ent.*<sup>2-6</sup>) have been sketched in upon the left side to serve as landmarks and to show the relations of the nerves to the thoracic appendages. The positions of the three sensory knobs are indicated by the enclosed areas at their outer extremities. The positions of the abdominal appendages are indicated by the external branchial muscles (*e.b.m.*<sup>8-13</sup>), the branchial cartilages (*b.c.*<sup>8-13</sup>), the tendinous stigmata (*t.s.*<sup>8-13</sup>), and the abdominal endochondrites (*a.e.*<sup>8-13</sup>). In the cephalothorax (*pros.*) all the tergo-coxal and plastro-coxal muscles have been dissected away, leaving the endocranium (*endo.*) with the occipital ring (*oc.r.*) exposed. Upon the left side of the animal one of the tergo-propastral muscles (*t.p.m.a*) is represented, and the branchio-thoracic muscles (*b.t.m.*) are seen extending into the cephalothorax. The longitudinal abdominal muscles (*l.a.m.*) are seen in the abdomen. Upon the right side of the animal all the muscles have been omitted except the haemo-neural muscles (*h.n.m.*<sup>8-14</sup>). The last two haemo-neural muscles (*h.n.m.*<sup>13</sup> and <sup>14</sup>) are represented upon the left side also. The large entapophysis (*enta.*<sup>7</sup> and <sup>8</sup>) of the cephalothorax and the smaller ones (*enta.*<sup>9-14</sup>) of the abdomen are shown upon the right side. At the base of the telson (*tel.*) the flexors (*t.f.m.*) and extensors (*t.e.m.b*) of the caudal spine are represented as cut off near their insertions. The anal muscles, sphincter ani (*s.a.*), levator ani (*l.a.*), and occludor ani (*o.a.*), and their relations to the anus (*a.*) are shown in the same region.

The oesophagus (*oe.*) runs forward upon the neural side of the endocranium to the proventriculus (*prov.*). From this the intestine (*int.*) passes posteriorly on the haemal side of the endocranium, and emerges upon the posterior side of this structure, whence it may be traced to the anus.

The brain lies upon the neural side of the endocranium, and the ventral cord (*v.c.*) passes back through the occipital ring (*oc.r.*), haemal to the abdominal endochondrites (*a.e.*<sup>8-13</sup>). All of the neural nerves (*n.n.*<sup>1-16</sup>) are cut off, but the haemal nerves (*h.n.*<sup>1-16</sup>) upon the left side are represented entire, as are also the nerves from the fore-brain (*f.br.*).

The first pair of neural nerves (*n.n.*<sup>1</sup>) go to the chelicerae. The second to sixth pairs go to the next five thoracic appendages, which are represented by the entocoxites (*ent.*<sup>2-6</sup>). The seventh pair of neural nerves (*n.n.*<sup>7</sup>) go to the chilaria, and the eighth pair (*n.n.*<sup>8</sup>) to the operculum. The two latter pairs pass through the occipital ring. The neural nerves from the ninth to the thirteenth (*n.n.*<sup>9-13</sup>) arise from the abdominal ganglia and innervate the five pairs of gills.

From the fore-brain a median olfactory nerve (*m.ol.n.*) and two lateral ones (*l.ol.n.* and *r.ol.n.*) pass forward to the olfactory organ; a median eye nerve (*m.ey.n.*) passes anteriorly and haemally upon the right of the proventriculus (*prov.*) to the median eyes; and a pair of lateral eye nerves (*l.e.n.*) sweep around the outer extremities of the entocoxites (*ent.*<sup>2-4</sup>) to the lateral eye (*l.e.*) upon the haemal side of the lateral expansion of the carapace.

The first haemal nerve, or lateral nerve (*l.n.*), follows the general course of the lateral eye nerve, but continues posteriorly far back onto the neural side of the abdomen.

The haemal nerves of the hind-brain (*h.n.*<sup>2-6</sup>) radiate from the brain to the margins of the prosomatic carapace, and each one passes anterior to the appendage of its own metamere. The integumentary portions divide into haemal and neural branches of which the haemal branches (*h.*) are cut off. Each haemal branch gives off a small nerve, which turns back toward the median line upon the haemal side of the body.

The haemal nerves (*h.n.*<sup>7 and 8</sup>) of the accessory brain pass through the occipital ring and out to the sides of the body between the operculum and the sixth thoracic appendage. The seventh innervates the posterior angles of the cephalothorax, the eighth the opercular portion of the abdomen.

The next five haemal nerves (*h.n.*<sup>9-13</sup>) arise from the five branchial neuromeres and pass out anterior to the five pairs of gills to the sides of the abdominal carapace and innervate the first five spines (*a.s.*<sup>9-13</sup>) upon the sides of the abdomen.

The first post-branchial nerve (*h.n.*<sup>14</sup>) innervates the last abdominal spine (*a.s.*<sup>14</sup>); the second post-branchial nerve (*h.n.*<sup>15</sup>) and one branch of the third post-branchial (*h.n.*<sup>16</sup>) innervate the muscles of the telson and the posterior angles of the abdomen; and the caudal branch of the third post-branchial nerve innervates the telson.

Intestinal branches (*i.n.*<sup>6-16</sup>) arise from all the haemal nerves from the sixth to the sixteenth and pass to the longitudinal abdominal muscles and to the intestine. Those from the sixth and seventh neuromeres (*i.n.*<sup>6 and 7</sup>) pass through foramina in the endocranium.

Cardiac nerves (*s.c.n.*<sup>6-13</sup>) arise from all the haemal nerves from the sixth to the thirteenth. Those which arise from the seventh and eighth neuromeres (*i.n.*<sup>7 and 8</sup>) fuse together. Six of the cardiac nerves (*s.c.n.*<sup>8-13</sup>) communicate with a lateral nerve, which has been called the lateral sympathetic nerve (*l.s.n.*). A branch (*x.*) from the fifth haemal nerve (*h.n.*<sup>5</sup>) may also be a cardiac branch.

The lateral sympathetic nerve (*l.s.n.*) innervates the branchio-thoracic muscles (*b.t.m.*). It receives in each metamere, from the eighth to the fourteenth, a branch from either the haemal or the cardiac nerve.

Two post-cardiac nerves (*s.c.n.*<sup>14 and 15</sup>) arise from the first two post-branchial nerves and pass to the haemal side of the body, where they anastomose with a branch from the last cardiac nerve (*s.c.n.*<sup>13</sup>) and innervate the extensors (*t.e.m.a.*) of the telson, and the epidermis posterior to the heart.

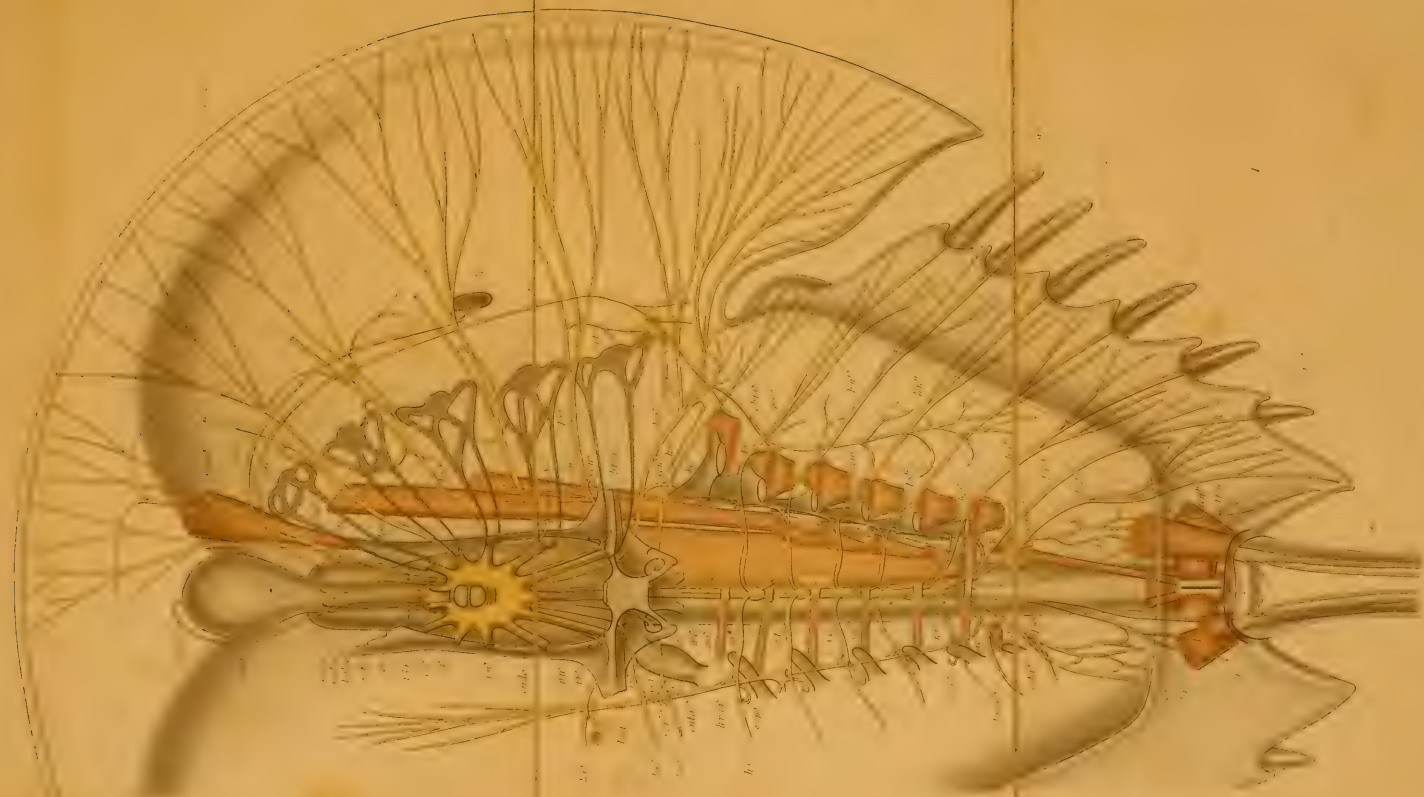


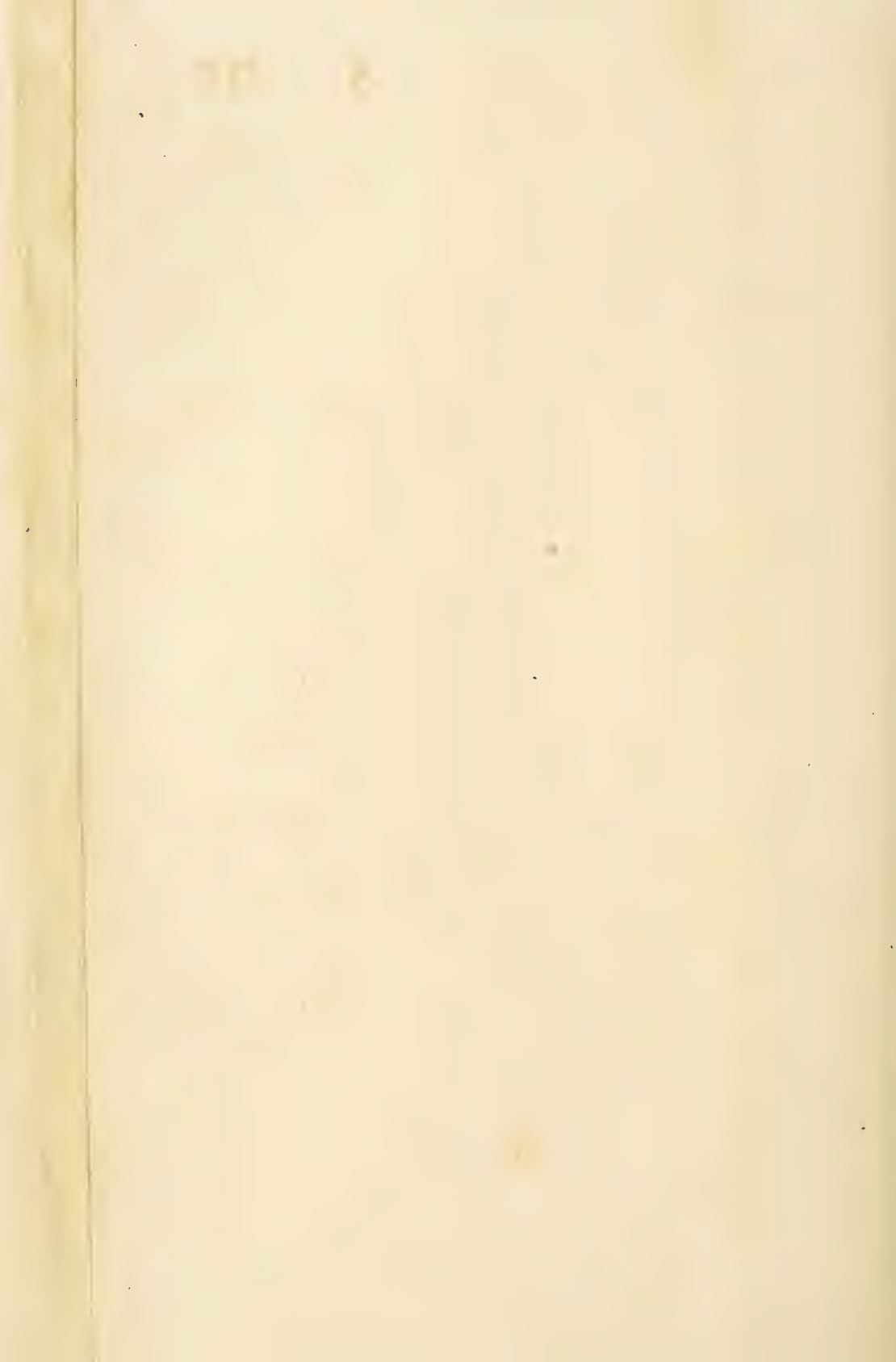












## EXPLANATION OF PLATE VII.

FIG. 2. A portion of the cephalothorax, showing the brain and the relations of the nerves to the tergo- and plastro-coxal muscles, sensory knobs, etc., in the region of the entocoxites (seen from the neural side and magnified about one and one-half times).

The plastro-coxal muscles ( $\mathcal{A}^a, b, e$ , and  $g$ ,  $\mathcal{B}^a, b, e, f$ , and  $g$ , etc.) hide the whole of the endocranium except the occipital ring ( $oc.r.$ ) and the capsuliginous bars ( $b.c.^7$ ).

The outer portions of the entocoxites ( $ent.^{2-6}$ ) are indicated with attached tergo-coxal muscles ( $\mathcal{A}^c, d, h, i$ , and  $j$ ,  $\mathcal{B}^c, d, i$ , and  $j$ , etc.).

The three areas enclosed by the outer portions of the entocoxites represent the three sensory knobs. The base of one of the chelicerae, with the attached tergo-coxal muscles ( $r$ ) of that appendage, and the bases of the chilaria, with some of the chilial muscles ( $\gamma^a$  and  $\gamma^c$ ), are also shown.

The four lobes of the nephridia ( $n.^{2-5}$ ), with connecting ducts and nephridial opening ( $n.o$ ), are represented lying between the plastro-coxal muscles of the anterior and posterior sides of the second to fifth thoracic appendages.

The brain lies neural to the plastro-coxal muscles; and the ventral cord ( $v.c.$ ), with the nerves from the accessory brain, passes out through the occipital ring ( $oc.r.$ ). The anterior commissure ( $a.c.$ ), with the three rostral nerves ( $la.n.$ ), is situated in front of the aperture through which the oesophagus passes. Four of the posterior commissures are also seen.

Upon the left side of the animal the neural nerves ( $n.n.^{2-6}$ ) of the hind-brain are cut off close to the brain to show the ento-coxal branches underneath. The cheliceral nerves ( $n.n.^4$ ) turn forward over the fore-brain ( $f.br.$ ). The chilial ( $n.n.^7$ ) and opercular ( $n.n.^8$ ) nerves proceed from the posterior side of the brain through the occipital ring ( $oc.r.$ ).

From the fore-brain the three olfactory nerves ( $ol.n.$ ) pass forward to the olfactory organ ( $ol.or.$ ); a portion of the median eye nerve ( $m.ey.n.$ ) is also represented; and the lateral eye nerve ( $l.e.n.$ ) sweeps anteriorly around the entocoxites ( $ent.^2$ ) to the lateral eye ( $l.e.$ ).

The delicate lateral nerve ( $l.n.$ ), or first haemal nerve, runs parallel to the lateral eye nerve and at one point fuses with the second haemal nerve ( $h.n.^2$ ). It is continued posteriorly onto the abdomen.

The other haemal nerves ( $h.n.^{2-8}$ ) radiate from the haemal side of the brain and pass to the sides of the carapace, each one anterior to the appendage of its own metamere. Each divides into a haemal ( $h.$ ) and a neural branch, and in the four anterior nerves the haemal branch passes haemal to the lateral eye nerve. A small branch turns backward toward the median line and innervates a portion of the epidermis of the haemal side of the carapace. The haemal nerves ( $h.n.^7$  and  $8$ ) of the accessory brain pass posteriorly through the occipital ring and out toward the sides of the body.

Intestinal branches ( $i.n.^{6-8}$ ) arise from the sixth, seventh, and eighth haemal nerves, but the one from the eighth ( $i.n.^8$ ) is concealed by the overlying chilial muscles.

Cardiac branches ( $s.c.n.^{6-8}$ ) also arise from the sixth, seventh, and eighth haemal nerves; those from the seventh and eighth ( $i.n.^7$  and  $8$ ) fuse together, and the

cardiac root (*s.c.n.*<sup>8</sup>) of the eighth gives a branch to the lateral sympathetic nerve (*l.s.n.*). A branch (*x.*), which may be either a cardiac or an intestinal nerve, arises from the fifth haemal nerve (*h.n.*<sup>5</sup>).

In the third, fourth, and fifth appendages there are three ento-coxal branches of the neural nerve; an anterior one (*a.e.n.*), innervating the muscles of the anterior side of the entocoxite and the anterior sensory knob; a posterior one (*p.e.n.*), innervating the muscles of the posterior side of the entocoxite and the posterior sensory knob; and a median one, going to the median (*m.e.n.*) sensory knob. The median one arises from the neural nerve at some distance from the brain, and hence is represented with its proximal end cut off.

In the second appendage only the anterior and posterior ento-coxal nerves (*a.e.n.*<sup>2</sup> and *p.e.n.*<sup>2</sup>) have been found.

In the sixth appendage the median sensory knob is replaced by the flabellum, and the median ento-coxal nerve (*m.e.n.*<sup>6</sup>) becomes the flabellar nerve. This is also cut off at its proximal end.











## EXPLANATION OF PLATE VIII.

FIG. 3. A sagittal section of *Limulus*, showing the nerves and principal organs in relief (seen from the right side, somewhat larger than natural size).

All the prosomatic appendages (*ap.*<sup>2-6</sup>), except the chelicera (*ap.*<sup>1</sup>) and chilarium (*ap.*<sup>7</sup>) of the right side, are omitted. The operculum (*ap.*<sup>8</sup>) and the five gills (*ap.*<sup>9-13</sup>) are represented in the abdominal region.

All the muscles are also omitted except the fibers running from the occipital ring (*oc.r.*) to the posterior side of the oesophagus (*oe.*), the chilarial muscles (*7c.*), the sphincter ani (*s.a.*), and the levator ani (*l.a.*).

The endocranium (*endo.*), with the occipital ring (*oc.r.*) and the capsuliginous bar (*b.c.*<sup>7</sup>), is seen from the side, and the positions of the abdominal endochondrites (*a.e.*<sup>8-13</sup>) are indicated.

The mouth (*m.*) leads into the oesophagus (*oe.*), which passes through the brain and forward to the proventriculus (*prov.*). A constriction, which marks the position of the pyloric valve, separates the proventriculus from the intestine (*int.*) which passes posteriorly to the anus (*a.*). A pair of hepatic ducts (*h.d.*<sup>a</sup> and *b*) enter the intestine opposite the endocranium.

The heart (*ht.*), surrounded by the pericardial sinus (*p.s.*), lies haemal to the intestine. The pericardium (*p.*) is shown between the heart and the intestine. The ostia (*os.*<sup>6-13</sup>) of the heart and the origins of the four lateral arteries (*l.ar.*<sup>6-9</sup>) are indicated upon the sides of the heart; the frontal artery (*f.ar.*) and the aortic arches (*ao.a.*), curving down to the brain, arise from the anterior end of the heart; the superior abdominal artery (*s.a.ar.*), and the opening of the collateral artery into it, are seen at the posterior extremity of the heart.

The brain surrounding the oesophagus is seen in side view upon the neural side of the endocranium. The ventral cord (*v.c.*) passes through the occipital ring (*oc.r.*) into the abdominal region. The anterior commissure (*a.c.*), with the three rostral nerves (*la.n.*) innervating the rostrum, or labrum (*la.*), and four of the post-oral commissures are represented.

The cheliceral nerve (*n.n.*<sup>1</sup>) with the small external pedal branch is shown entire, but the next five neural nerves (*n.n.*<sup>2-6</sup>) are cut off. The chilarial nerve (*n.n.*<sup>7</sup>), the opercular nerve (*n.n.*<sup>8</sup>), and the five branchial nerves (*n.n.*<sup>9-13</sup>) enter their respective appendages, the two former (*n.n.*<sup>7</sup> and <sup>8</sup>) passing through the occipital ring.

From the fore-brain the three olfactory nerves (*ol.n.*) pass anteriorly to the olfactory organ (*ol.or.*); the median eye nerve (*m.ey.n.*) passes to the right of the proventriculus (*prov.*) to the median eyes (*m.e.*); the lateral eye nerve (*l.e.n.*) passes forward and is represented as cut off opposite the proventriculus. The lateral nerve (*l.n.*), or first haemal nerve, is also cut off just beyond the point where it fuses with the second haemal nerve (*h.n.*<sup>2</sup>). The stomodaeal nerve (*st.n.*) ramifies over the oesophagus and proventriculus.

The second haemal nerve (*h.n.*<sup>2</sup>) passes to the anterior extremity of the carapace; its haemal branch is cut off opposite the proventriculus. An intestinal branch (*i.n.*<sup>2</sup>) arises from near its base and disappears behind the anterior cornu of the endocranium.

The next three haemal nerves (*h.n.*<sup>3-5</sup>) are cut off close to the brain, and the

following nine haemal nerves (*h.n.*<sup>6-14</sup>) are cut off beyond the cardiac branches. The second post-branchial nerve (*h.n.*<sup>15</sup>) is cut off beyond its branch to the telson muscles. Both branches of the haemal nerve (*h.n.*<sup>16</sup>) are represented extending into the telson (*tel.*).

The intestinal nerves (*i.n.*<sup>6-16</sup>) are shown arising from the haemal nerves and entering the intestine. Those from the sixth and seventh neuromeres (*i.n.*<sup>6</sup> and <sup>7</sup>) pass through foramina in the endocranium and communicate with a plexus in the longitudinal abdominal muscles before entering the intestine. The eighth passes just posterior to the endocranium and joins the same plexus. Those from the first four branchial neuromeres (*i.n.*<sup>9-12</sup>) arise very near the abdominal ganglia and are double in their origins, the anterior branches joining the above-mentioned plexus, and the posterior branches entering the intestine. The thirteenth, fourteenth, and fifteenth are somewhat complicated in their relations and will be described to better advantage under Fig. 4. The fifteenth extends far back towards the rectum and anastomoses with the sixteenth (*i.n.*<sup>16</sup>), which arises from the caudal branch of the sixteenth haemal nerve (*h.n.*<sup>16</sup>) and innervates the rectum and anal muscles.

The segmental cardiac nerves (*s.c.n.*<sup>6-13</sup>) arise from the haemal nerves of the sixth to the thirteenth neuromeres respectively. The most anterior one (*s.c.n.*<sup>6</sup>) passes haemally to the inter-tergal muscles and epidermis in median line, haemal to the heart, but the connections with the cardiac plexus have not been made out. The next two (*s.c.n.*<sup>7</sup> and <sup>8</sup>) fuse to form a large nerve, which likewise passes haemal to the heart, to the inter-tergal muscles, and epidermis, but has not been observed to connect directly with the cardiac plexus. It, however, sends posteriorly a branch, the pericardial nerve (*p.n.*), which in turn gives a branch to each of the cardiac nerves of the branchial neuromeres (*s.c.n.*<sup>9-13</sup>), and then continues onward to the posterior margin of the abdomen. This nerve lies in the epidermis haemal to the heart. The median and lateral cardiac nerves (*m.c.n.* and *l.c.n.*) are seen upon the walls of the heart. The five cardiac nerves (*s.c.n.*<sup>9-13</sup>) from the branchial neuromeres pass haemal to the heart, in the epidermis, to the median line, and dip down to the median nerve (*m.c.n.*) of the heart opposite the last five pairs of ostia (*o.s.*<sup>9-13</sup>). They communicate with the pericardial nerve (*p.n.*) and also with the lateral sympathetic nerve (*l.s.n.*).

Two post-cardiac nerves (*s.c.n.*<sup>14</sup> and <sup>15</sup>) pass from the first and second post-branchial nerves (*h.n.*<sup>14</sup> and <sup>15</sup>) to the epidermis posterior to the heart.

The last cardiac nerve (*s.c.n.*<sup>13</sup>) and the two post-cardiac nerves (*s.c.n.*<sup>14</sup> and <sup>15</sup>) give off branches which anastomose with each other and innervate the extensors of the telson.

The lateral sympathetic nerve (*l.s.n.*) receives branches from all the neuromeres from the eighth to the fourteenth, either through the cardiac nerves or the haemal nerves, and innervates the branchio-thoracic muscles, extending with these far into the cephalothorax.

FIG. 4. This drawing is intended to show the intestinal nerves from the haemal side, and is made to the same scale as Fig. 3. Most of the heart and a large portion of the intestine have been removed, leaving exposed the haemal sides of the endocranium, ventral cord, muscles inserted on the endocranium, and the plexus of intestinal nerves.

The anterior end of the heart (*ht.*) with the aortic arches (*ao.a.*) and frontal

artery (*f.ar.*), the anterior portion of the intestine (*int.*) with proventriculus (*prov.*) and oesophagus (*oe.*), and the rectum, or proctodaeum (*proc.*), with the sphincter ani (*s.a.*) and levator ani (*l.a.*), are left in position.

The muscles are dissected away from the left side of the endocranium (*endo.*), so that the various parts are exposed to view, *viz.*, anterior cornu (*a.cor.*), lateral cornu (*l.c.*), haemal processes (*h.pr.*), latero-posterior processes (*l.p.pr.*), posterior process (*p.pr.*), capsuliginous bars (*b.c.*<sup>7</sup>), and the foramina (*f.*<sup>6</sup> and *f.*<sup>7</sup>).

Some of the abdominal endochondrites (*a.e.*<sup>8-13</sup>) with the attached haemo-neural muscles (*h.n.m.*<sup>8-14</sup>) are also seen.

To the anterior cornu (*a.cor.*) of the endocranium are attached three tergo-pro-plastral muscles (*t.p.m.*<sup>a-c</sup>); to the haemal process (*h.pr.*), the dorso-lateral plastro-tergal muscles (*d.l.p.t.*) and the dorso-lateral plastro-entapophysial muscle (*d.l.p.e.*); from the side of the posterior process a large meso-plastro-entapophysial (*m.p.e.*) muscle goes to the large entapophysis (*enta.*<sup>7</sup> and <sup>8</sup>); longitudinal abdominal muscles (*l.a.m.*) go from the endocranium to each of the abdominal entapophyses (*enta.*<sup>9-14</sup>); and inter-entapophysial muscles (*i.e.m.*) go from the first entapophysis (*enta.*<sup>7</sup> and <sup>8</sup>) to the next four (*enta.*<sup>9-12</sup>).

Two veno-pericardiac muscles (*v.p.m.*<sup>6</sup> and <sup>7</sup>) are attached to the sides of the endocranium and the bases of the remaining six (*v.p.m.*<sup>8-13</sup>) are seen amongst the longitudinal abdominal muscles.

The ventral cord (*v.c.*) emerges from behind the endocranium and presents to view the abdominal ganglia with the roots of the neural and haemal nerves posterior to the eighth neuromere. The neural nerves (*n.n.*<sup>9-13</sup>) are cut off. The haemal nerves upon the left are cut off, and those upon the right are all concealed from view except the last four (*h.n.*<sup>13-16</sup>), but their bases with the origins of the intestinal nerves are exposed.

The plexus of intestinal nerves upon the abdominal muscles is in life largely hidden from view within these muscles. All the branches which go to the intestine are represented as cut off at the points where they enter the organ.

The two anterior intestinal nerves (*i.n.*<sup>6</sup> and *i.n.*<sup>7</sup>) come through the foramina (*f.*<sup>6</sup> and *f.*<sup>7</sup>), and these with the next one (*i.n.*<sup>8</sup>) join the plexus within the muscles upon the haemal side of the endocranium. From this plexus numerous branches run forward to the anterior part of the intestine.

In the abdominal region some regularity about the branching of the intestinal nerves has been observed. In the ninth, tenth, and eleventh neuromeres two intestinal branches (*a.i.n.* and *p.i.n.*) arise; the anterior one (*a.i.n.*) joins the plexus in the abdominal muscles; the posterior one (*p.i.n.*) gives a branch to the haemo-neural muscle (*h.n.m.*) of its own neuromere and a branch to the intestine.

In the twelfth neuromere the posterior branch anastomoses with the plexus in the abdominal muscles, sends one branch to the haemo-neural muscle (*h.n.m.*<sup>12</sup>), whence one proceeds to the intestine. It also gives off another posteriorly which anastomoses with a branch from the fourteenth haemal nerve, and then proceeds to the intestine.

From the thirteenth haemal nerve (*h.n.*<sup>13</sup>) only one intestinal branch (*i.n.*<sup>13</sup>) arises, and this enters the intestine by two branches.

From the fourteenth haemal nerve (*h.n.*<sup>14</sup>) upon the left side an intestinal nerve (*i.n.*<sup>14</sup>) arises and, passing a long distance posteriorly, enters the intestine by several branches. From the fourteenth haemal nerve upon the right side three

nerves arise; the first one anastomoses with the twelfth intestinal nerve; the second enters the longitudinal abdominal muscles; and the third enters the intestine.

From the fifteenth haemal nerve (*h.n.*<sup>15</sup>) an intestinal branch (*i.n.*<sup>15</sup>) arises, which goes posteriorly close to the intestine, gives several branches to this organ, and then anastomoses with the intestinal branch (*i.n.*<sup>16</sup>) of the last neuromere.

The last intestinal branch (*i.n.*<sup>16</sup>) innervates the rectum and anal muscles (*l.a.* and *s.a.*).





## EXPLANATION OF PLATE IX.

FIG. 5. The heart of *Limulus*, with adjoining organs and nerves, seen from the haemal side (drawn to same scale as Figs. 3 and 4).

The haemal side of the carapace has been stripped off and the epidermis and median inter-tergal muscles removed in order to show more clearly the heart and arteries. The nerves, however, lying within these omitted portions are represented.

The heart (*ht.*) lies in the pericardial sinus, which is indicated by the shaded area upon each side of the organ. Eight pairs of ostia (*os.*<sup>6-13</sup>) are seen upon the haemal side of the heart, and the median (*m.c.n.*) and two lateral cardiac nerves (*l.c.n.*) are indicated. The striated appearance of the walls of the heart is due to the longitudinal strands of connective tissue.

Upon the left side of the heart the alary muscles (*al.m.*<sup>6-13</sup>) are represented, but upon the right they are omitted in order to show the four lateral arteries (*l.ar.*<sup>6-9</sup>) and the positions of the collateral arteries (*c.ar.*), which unite posterior to the heart to form the superior abdominal artery (*s.a.ar.*). Another lateral artery (*l.ar.*<sup>5</sup>) sometimes arises from the anterior end of the heart in front of the aortic valve. Upon the outer sides the collateral arteries give off numerous branches to the muscles and haemal side of the body, and upon the median sides they give branches to the intestine, the superior intestinal branches (*s.i.ar.*<sup>12</sup>). The aortic arches (*ao.a.*) and frontal artery (*f.ar.*), running over the proventriculus (*prov.*), proceed from the anterior extremity of the heart.

Five pairs of branchio-cardiac canals bring blood to the pericardial sinus; the first of these is formed of two canals, one (*b.c.c.*<sup>8</sup>) from the operculum and the other (*b.c.c.*<sup>9</sup>) from the first gill. The remaining four pairs of branchio-cardiac canals (*b.c.c.*<sup>10-13</sup>) bring the blood from the last four pairs of gills.

The seven pairs of entapophyses (*enta.*<sup>7-14</sup>) are represented, and the principal muscles attached to the haemal side of the carapace; three tergo-proplastral muscles (*t.p.m.*<sup>a-c</sup>), two slips of the branchio-thoracic muscles (*b.t.m.*<sup>a</sup> and <sup>b</sup>), the dorso-lateral-plastro-tergal muscle (*d.l.p.t.*), and a slip of the inter-tergal muscle (*i.m.*) attached to the first entapophysis (*enta.*<sup>7</sup> and <sup>8</sup>) are represented in the cephalo-thoracic region; in the abdominal region are seen the haemal ends of the seven pairs of haemo-neural muscles (*h.n.m.*<sup>8-14</sup>) and the six external branchial muscles (*e.b.m.*<sup>8-13</sup>) of the right side, the levator ani (*l.a.*) and the extensors (*t.e.m.*<sup>a</sup> and <sup>b</sup>) of the telson. One slip of the extensor (*t.e.m.*<sup>a</sup>) is attached to the last three pairs of entapophyses (*enta.*<sup>12-14</sup>).

The end of the median eye nerve (*m.ey.n.*) appears in front of the proventriculus.

Seven pairs of segmental cardiac nerves (*s.c.n.*<sup>6-13</sup>) and two post-cardiac nerves (*s.c.n.*<sup>14</sup> and <sup>15</sup>) come from the neural side of the body and pass up into the epidermis haemal to the heart. The most anterior one (*s.c.n.*<sup>6</sup>) supplies the omitted inter-tergal muscle, and also a portion of the epidermis. The next one (*s.c.n.*<sup>7</sup> and <sup>8</sup>) is formed of the fused branches of the seventh and eighth neuromeres; this also innervates the above-mentioned inter-tergal muscles and sends branches to the epidermis in the median line; one branch, the pericardial nerve (*p.n.*), goes posteriorly in the epidermis, and gives a branch to each of the branchial cardiac



## EXPLANATION OF PLATE X.

FIG. 9. A portion of the cardiac plexus of Fig. 8 enlarged 40 diameters.

The masses of ganglion cells (*g.c.*) in the median nerve (*m.c.n.*) are more distinct, and the courses of the individual fibers in the plexus and lateral nerve (*l.c.n.*) are more apparent. Two of the ostia (*os.* 7 and 8) are represented.

FIG. 10. A small portion of the median cardiac nerve (*m.c.n.*), enlarged 500 times.

The individual nerve fibers and the ganglion cells (*g.c.*), with their processes, are easily made out.

FIGS. 11 AND 12. The brain of *Limulus* from the neural and haemal sides, respectively (enlarged about 3 diameters).

The enveloping arterial sheath has not been removed. The anterior commissure (*a.c.*) with the three rostral nerves (*l.a.n.*) is seen in Fig. 11, behind the fore-brain (*f.br.*), and four of the post-oral commissures (*p.o.c.* 1-5) can be made out posterior to the central canal through which the oesophagus passes. The first post-oral commissure (*p.o.c.* 1) is separated from the others, and is much longer as it passes haemal to the oesophagus.

Three olfactory nerves (*ol.n.*) arise from the anterior side of the fore-brain, and a median eye nerve (*m.e.y.n.*) comes through the arterial sheath a little to one side of the median line and near the haemal surface. From the haemal side of the fore-brain arise the two large lateral eye nerves (*l.e.n.*) with ganglionated bases (Fig. 12).

Just back of the fore-brain upon the neural side, the cheliceral nerves (*n.n.* 1, Fig. 11) arise and give off, near their bases, from one to three small nerves to the tergo-coxal muscles of the chelicerae.

Five more pairs of large neural nerves (*n.n.* 2-6) radiate from the circum-oesophageal collar to the next five pairs of thoracic appendages. Each of these gives off on the neural side a mandibular branch (*m.n.* 2-6) and upon the haemal side several ento-coxal nerves. The typical number of ento-coxal nerves is three; an anterior (*a.e.n.* 3), a posterior (*p.e.n.* 3), and a median (*m.e.n.* 3) ento-coxal nerve. From the second haemal nerve (*h.n.* 2) four ento-coxal branches are given off, in some cases; but two of these may be regarded as branches of the anterior and posterior ento-coxal nerves; the median ento-coxal nerve has not been found. In the case of the sixth neural nerve (*n.n.* 6) the median ento-coxal nerve (*m.e.n.* 6) is much enlarged and becomes the flabellar nerve.

The chilarial (*n.n.* 7) and opercular nerves (*n.n.* 8) are much smaller than the other neural nerves, and arise from the posterior side of the brain (Fig. 11) neural to the ventral cord (*v.c.*).

From the haemal side of the brain (Fig. 12) the delicate lateral nerves (*l.n.*), or first pair of haemal nerves, arise just back of the lateral eye nerves (*l.e.n.*).

The next seven pairs of haemal nerves (*h.n.* 2-8) radiate from the circum-oesophageal collar. Of these the anterior pair (*h.n.* 2) are somewhat larger than the others and give off close to the brain a small nerve (*i.n.* 2) which innervates the tergo-proplastral muscles.

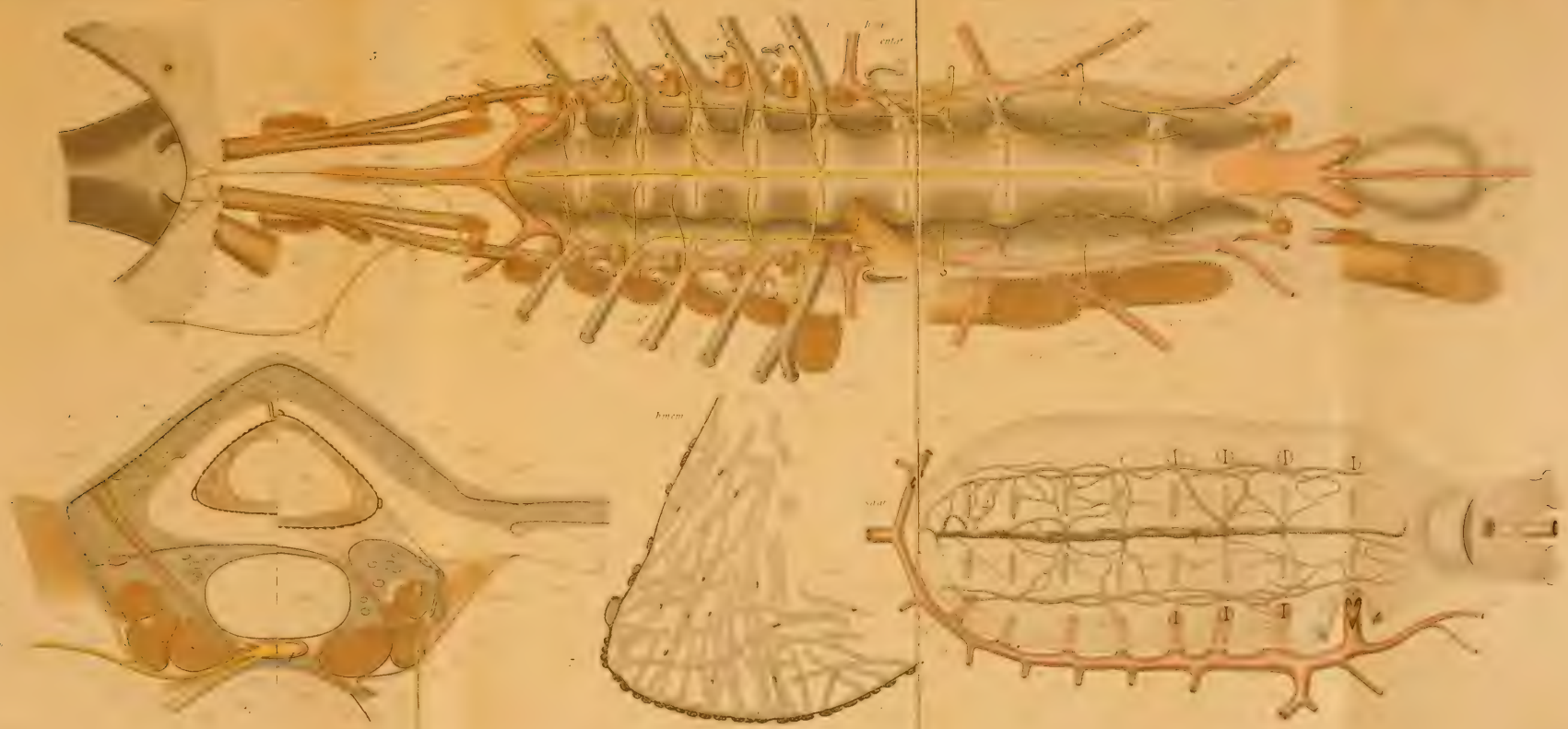
The posterior three pairs (*h.n.* 6-8) give off intestinal nerves (*i.n.* 6-8), two of which (*i.n.* 6 and 7) go through foramina in the endocranium.

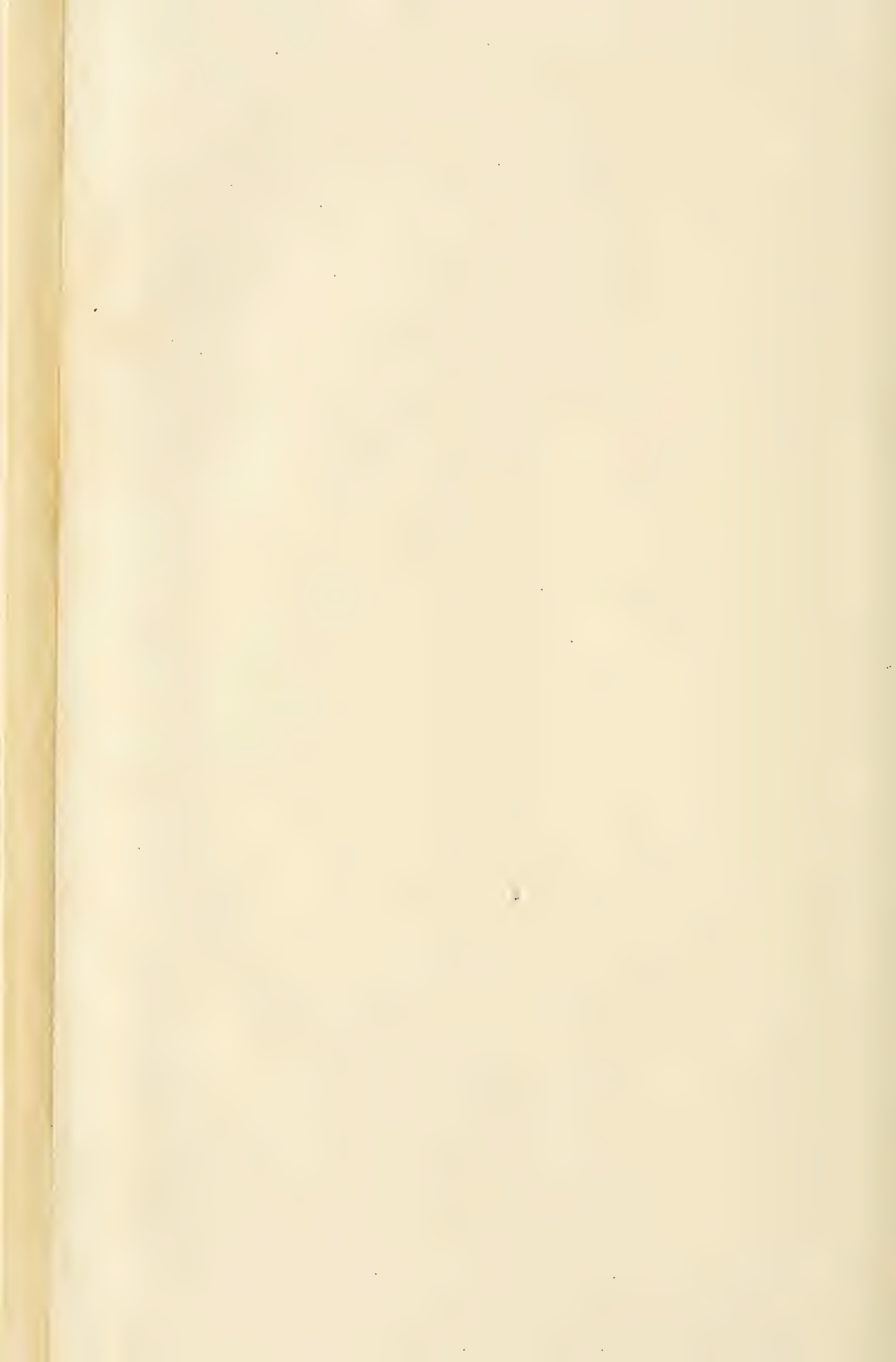
A pair of stomodaeal nerves (*st.n.*) arise from small ganglia upon the inner side of the circum-oesophageal collar. Sometimes small nerves are given off to the oesophagus from the stomodaeal ganglia.











nerves (*s.c.n.*<sup>9-13</sup>). The pericardial nerve also gives a branch to the inter-tergal muscle (*i.m.*) which is attached to the first entapophysis. These two anterior nerves (*s.c.n.*<sup>6</sup> and *s.c.m.*<sup>7 and 8</sup>) have not been observed to connect with the cardiac plexus.

The cardiac nerves (*s.c.n.*<sup>9-13</sup>) from the five branchial neuromeres send numerous branches to the epidermis, and also branches to the median cardiac nerve (*m.c.n.*) opposite each of the last five pairs of ostia (*os.*<sup>9-13</sup>). There is considerable variation in the arrangement of the nerves in the posterior portion of the haemal region. Moreover, the branches are very numerous and very fine, so that many of them are necessarily missed in dissection. Some of the branches are continued posteriorly to the margin of the carapace.

The two post-cardiac nerves (*s.c.n.*<sup>14 and 15</sup>) send branches in the epidermis to the haemal line. Branches from the last cardiac (*s.c.n.*<sup>13</sup>) and from the two post-cardiac nerves (*s.c.n.*<sup>14 and 15</sup>) anastomose, and innervate one of the slips of the extensor (*t.e.m.a*) of the telson. Other branches of the last post-cardiac nerve innervate the other extensor (*t.e.m.b*) and the epidermis of the posterior margin of the abdomen.

FIG. 6. A vertical section through the abdominal region in the ninth and tenth neuromeres showing the course of one of the cardiac nerves (from the anterior side, about twice natural size).

The left half of the drawing represents the posterior portion of the ninth neuromere, and the right half the anterior portion of the tenth neuromere.

The heart (*ht.*) is seen in cross-section showing a central lumen surrounded by a layer of anastomosing muscle fibers; the median and lateral cardiac nerves (*m.c.n.* and *l.c.n.*) are shown in the three angles of the heart.

Upon the right, one of the alary muscles (*al.m.*<sup>10</sup>) runs from the lateral angle of the heart to the pericardium.

The pericardial sinus (*p.s.*) surrounds the heart and the branchio-cardiac canal (*b.c.c.*<sup>10</sup>) is seen coming from the upper portion of the second gill (*ap.*<sup>10</sup>).

A pericardial membrane (*p.*) bounds the pericardial sinus upon the neural side. It is attached to the entapophysis (*enta.*<sup>9</sup>) upon the left and to the branchial cartilage (*b.c.*<sup>10</sup>) upon the right. The veno-pericardiac muscles (*v.p.m.*<sup>10</sup>) are attached to it, and the collateral arteries (*c.ar.*) are found in close connection with it.

The intestine (*int.*) lies haemal to the ventral cord which is enclosed in the ventral artery (*v.ar.*).

Neural to the ventral cord is the abdominal endochondrite (*a.e.*<sup>9</sup>) which furnishes attachment for the haemo-neural muscles (*h.n.m.*<sup>9</sup>) and the internal branchial muscles (*i.b.m.*<sup>9</sup>). The external branchial muscles (*e.b.m.*<sup>9 and 10</sup>) are also seen, and the longitudinal abdominal muscles (*l.a.m.*) and branchio-thoracic muscles (*b.t.m.*) are cut across. The veno-pericardiac muscle (*v.p.m.*<sup>10</sup>) is seen attached to the pericardium, and spanning the venous-collecting sinus (*v.c.s.*) at its neural end.

An abdominal ganglion with its neural (*n.n.*<sup>9</sup>) and haemal nerves (*h.n.*<sup>9</sup>) is represented. The neural nerve and the integumentary portion (*in.n.*<sup>9</sup>) of the haemal nerve are cut off.

The intestinal nerves (*i.n.*<sup>9</sup>) arise from the haemal nerve close to the ganglion; the anterior branch (*a.i.n.*) goes to the longitudinal abdominal muscles (*l.a.m.*), and the posterior one (*p.i.n.*) goes to the haemo-neural muscle (*h.n.m.*<sup>9</sup>) and to the intestine.

The cardiac branch (*s.c.n.*<sup>9</sup>) arises opposite the branchio-thoracic muscle, and

gives a branch to the lateral sympathetic nerve (*l.s.n.*), and then continues through the pericardium to the areolar tissue and epidermis haemal to the heart. After giving off numerous branches to the epidermis, it dips down in the median line to the median cardiac nerve (*m.c.n.*).

FIG. 7. A cross-section of one corner of the heart of a young *Limulus* (magnified 100 times).

It shows the three layers of the heart; the layer of longitudinal connective-tissue fibers (*l.c.s.*) is seen in cross-section upon the outside of the hyaline basement membrane (*b.mem.*), and the layer of muscle fibers (*a.m.f.*) is seen upon the inside. These are cross-striated and anastomose freely. Their bases are expanded where they are attached to the basement membrane.

FIG. 8. Heart of a young *Limulus* (5 in. long). It has been slit open along the neural side, spread out and viewed from the interior (magnified 10 times).

The specimen, from which the drawing was made, was treated by Löwits's gold chloride method, which macerated off the muscles, leaving the nerve plexus intact.

The eight pairs of ostia (*os.*<sup>6-13</sup>) are indicated, and also the rudimentary ostia (*r.os.*) anterior to the aortic valve (*a.v.*). The two aortic arches (*ao.a.*) are slit open. The frontal artery, with its opening in front of the aortic valve, is seen between the aortic arches.

The lateral arteries (*lar.*<sup>6-9</sup>) emptying into the collateral artery (*c.ar.*) and the superior intestinal branches and superior abdominal artery (*s.a.ar.*) are also represented. The anterior lateral artery (*lar.*<sup>6</sup>) is cut open to show the two semi-lunar valves (*s.v.*<sup>6</sup>).

The median cardiac nerve (*m.c.n.*), with its blackened masses of ganglion cells, gives off branches opposite each pair of ostia to the plexus of nerves which communicates with the lateral cardiac nerves (*l.c.n.*).

the archiamphiaster stage the astrosphaeres of the egg of *Limax agrestis* conform very closely to the type of astrosphaere described by Van Beneden and by Boveri for *Ascaris*.

The centrosphere has a distinct peripheral zone, the "cortical" zone of Van Beneden, or "archoplasm" of Boveri, which stains intensely black with iron-haematoxylin. When stained with Lyons blue and borax-carminine it becomes intensely blue. This outer zone is often very sharply marked off from the rest of the cytoplasm, although the inner ends of the astral rays are in direct contact with its periphery (Pl. XI, Figs. 2, 3, and 6). On its inner circumference the outer "cortical" zone of the centrosphere is marked off with equal distinctness from a perfectly clear structureless zone, the "medullary" zone of Van Beneden. Sometimes, however, the concentric layers of the centrosphere are less sharply outlined than in Fig. 2, appearing as in Figs. 4 and 5. Figs. 4 and 5 represent sections through an egg that was killed immediately after it was laid. The egg is of approximately the same age as that represented in Fig. 6. The centrosphere in Figs. 4 and 5 is composed of four concentric rings, which are alternately dark and light, as in the centrospheres formed by only two rings. As the number of rings increases, the boundaries between them become less sharply defined. Here, as in Pl. XI, Figs. 2 and 3, the astral rays diverge from the periphery of the outermost dark ring or zone.

Within the clear inner zone of the centrosphere there is a deeply staining central mass, consisting of several granules that form the centrosome or centrosomes. These granules are often grouped irregularly, as in Fig. 6, but sometimes they are arranged so as to appear like two short thick rods in the form of dumb-bells (Pl. XI, Figs. 2 and 5). In haematoxylin the centrosomes stain almost black; in Lyons blue and borax-carminine they stain blue. The centers of the astrosphaeres in the archiamphiaster sometimes fail to show deeply staining central granules, but this is probably due either to the method of preservation or to some accident in staining, since the centrosomes are usually present even at a much earlier period. The inner



clear zone of the centrosphere, with its central granule or granules, corresponds to the centrosome of Boveri (Pl. XI, Figs. 2, 3, and 6).

## 2. *Ovarian Eggs.*

I have not attempted the study of the ovogenesis of *Limax*. My attention was directed to the ovarian eggs in the hope of finding in them the origin of the granular centrosomes of the archiamphiasier; for it was evident that the centrosomes appeared before the eggs were laid. I dissected a number of individuals and found several of them with eggs arranged in a row along the oviduct. The capsules had already been secreted about these eggs, which contained a well-formed amphiasier. Other individuals were dissected and were found to contain eggs in the lower part of the duct of the albuminous gland; these eggs had no capsules, but each egg contained an amphiasier. It was evident then that the origin of the centrosome must be looked for at a still earlier period.

I then sectioned the ovo-testis of *Limax* and found several eggs in the archiamphiasier stage. These eggs were in the lumen of a follicle that was filled with spermatozoa. They showed nothing of the origin of the centrosome, however, for the spindle was already fully formed (Pl. XI, Fig. 2). In Fig. 2 no trace of the germinal vesicle remains, the chromatin being already in the equatorial plate stage. Even at this early period the centrospheres are precisely like those of the newly laid ova; they have a clear central ("medullary") zone, in which two oblong deeply staining centrosomes are distinctly shown, and a peripheral ("cortical") zone which stains almost black with iron-haematoxylin.

The peripheral zone ("archoplasm" of Boveri) shows a very strong affinity for Lyons blue when stained with this dye and borax-carmin. Pl. XI, Fig. 1, shows a section of an ovarian egg in which the center of the aster is still undifferentiated. There is no distinct centrosphere or centrosome present, and the rays are short and relatively few in number. Unfortunately the series of sections through this egg is incomplete, so that I cannot make any statement in regard to the condition



of the nucleus, or in regard to the relation of the aster to the germinal vesicle.

Sections through the hermaphrodite gland show many large ovarian eggs which are still in contact with the walls of the follicles, and in which the germinal vesicles are evidently in various stages of breaking down. Even in the largest of these ova the germinal vesicle still retains a distinct membrane throughout the greater part of its circumference, and the large nucleolus, which is characteristic of all of the earlier stages of ovarian eggs, still persists.

In none of these ova is there any radiate structure present, or any evidence of the presence of a centrosome. The germinal vesicle must be lost and the archiamphiaster formed after the ovum becomes detached from the wall of the follicle; but as relatively few ovarian eggs reach maturity simultaneously in *Limax*, the different steps in the process of ripening are extremely difficult to find. Many of the younger ovarian eggs are oval in outline, with a large surface of attachment to the follicular wall. The structure of these ova is much denser immediately around the germinal vesicle than at the periphery of the cell. This dense region stains more deeply with haematoxylin than does the rest of the cytoplasm, and with Lyons blue and borax-carminé it takes a deep blue stain. The chromatin of the ovarian eggs also stains blue, as does likewise the chromatin in the head of the spermatozoa that are still in the follicles. These facts agree with Calkin's observations on the color reactions of chromatin and yolk-nuclei in the ovarian egg of *Lumbricus*. Beyond the fact that the chromatin and the cell substance surrounding the nucleus stain alike, however, I have no observations that indicate that the deeply dyed mass around the nucleus has been derived from the chromatin.

While the younger eggs are still oval in outline, the protoplasm is peculiarly stringy and irregular in appearance. Numerous bodies which appear circular in section, and which stain more deeply than the surrounding cytoplasm, are present at opposite poles of the long axis of the egg; there are also numerous clear spaces in the cytoplasm which resemble vacuoles. What the significance of these structures is, I have not

been able to determine ; they vary in size, number, and position, as do also the deeply staining bodies which probably correspond to the yolk-nuclei of other forms. As the ovarian eggs enlarge and leave the walls of the follicles, these structures disappear and the cytoplasm becomes more uniform in appearance.

3. *First Maturation Spindle. Extrusion of the First Polar Body.*

As long as the archiamphaster remains at the equator of the egg, the two polar asters are of the same size and retain the appearance already described. Within half an hour after the eggs have been deposited the archiamphaster, or the first maturation spindle, as it will be called henceforth, assumes a radial position at the upper pole of the egg. The arrangement of the centrosphere in concentric rings often persists until after the extrusion of the first polar body, as shown in Pl. XI, Figs. 9 and 10. Sometimes, however, before the first polar body is extruded, the centrosphere assumes a very different appearance. The two distinct zones that are so characteristic of the stage of the archiamphaster gradually fade away, and the center of the aster now appears as a deeply stained, homogeneous body, surrounded by a less deeply staining zone from which the astral rays diverge (Pl. XI, Figs. 7 and 8).

The astral rays in the eggs of *Limax* are formed by filaments which can be traced far out into the cytoplasm in almost straight lines (Pl. XI, Figs. 3, 7, and 8). During the stage of the first maturation spindle, Mark has found that the rays of the peripheral aster are slightly bent so as to form a spiral. I have never seen spiral asters in connection with the first maturation spindle in *Limax agrestis*.

The different forms under which the centrosphere of the first maturation spindle appears (Pl. XI, Figs. 7-9) may be due to different lengths of time elapsing between the formation of the archiamphaster and the extrusion of the first polar globule. The formation of the first polar body is preceded by a slight flattening of the upper pole of the egg. As the distal aster approaches the periphery of the egg, the rays are











at first pressed sideways against the upper pole (Pl. XI, Fig. 7), and as the polar globule is protruded, the rays are finally massed together to form a "Zwischenkörper" (Pl. XI, Fig. 8), which for a long time marks the place of extrusion of the first polar globule. The process of formation and extrusion of the polar body requires nearly two minutes for its completion. Soon after its separation from the egg the first polar globule collapses so that its position is marked only by a shriveled membrane at the outer periphery of the egg. Mark has described a similar behavior of the first polar globule in *Limax campestris*.

4. *Formation of the Second Maturation Spindle. Extrusion of the Second Polar Body.*

After the extrusion of the first polar globule the astrosphaere that remains in the egg undergoes a series of changes that are shown in Pl. XI, Figs. 10-14. The "cortical" and "medullary" zones of the centrosphere become less sharply defined as the sphaere continues to enlarge (Pl. XI, Figs. 12 and 14). The astral rays, which often persist for some time after the extrusion of the first polar globule, soon begin to shorten as if by contraction, so that the centrosphere is seen at one time surrounded by rays of uniform length, as in Pl. XI, Fig. 12, and again by still shorter rays that terminate at their inner ends at the periphery of the centrosphere, in a circle of granular thickenings or "microsomes" (Pl. XI, Fig. 13). Within the circle of microsomes the rays are continued centripetally as extremely delicate fibers that extend through the "cortical" zone to the "medullary" zone, which now appears slightly granular.

When the centrosphere reaches its maximum size it becomes uniformly very finely reticular, so that it appears almost granular and at the same time loses its strong affinity for staining reagents (Pl. XI, Fig. 14, *a, b, c*). In the center of the sphaere the centrosomes are often distinguishable as two tiny, deeply staining granules, as shown in Pl. XI, Figs. 14 *a* and 14 *c*. These centrosomes are evidently the same as those seen in the

archiamphiaster, but their form has changed. Between the centrosomes a light region (the "centrodesmose" of Heidenhain) sometimes marks the position of the central spindle, but at the time of the first appearance of the central spindle there are no distinct fibers visible (Pl. XI, Fig. 15).

While still within the sphere the centrosomes may become surrounded by a distinct peripheral zone that stains less deeply than the surrounding matrix. Sometimes no centrosomes are visible within the centrosphere at this stage, as in Pl. XI; Fig. 12; they may, however, be present. Soon after their separation the centrosomes in the centrosphere become the focal points from which new astral rays diverge (Pl. XI, Fig. 16). At the same time the rapidly fading rays of the old aster can be seen terminating in the peripheral ring of microsomes that surround the newly formed spindle. The rays of the new asters elongate rapidly and reach far out into the cells, so that at the equator of the spindle the rays from opposite poles meet and cross one another.<sup>1</sup>

At first the chromatin lies entirely outside the newly formed spindle, on the periphery of the centrosphere, where it was left after the extrusion of the first polar body. In the interval between the extrusion of the first and second polar globules no nuclear membrane is formed. These relations are shown in Pl. XI, Figs. 11, 14, and 15. As the second maturation spindle enlarges and the sphere in which it is first formed fades away, the chromatin comes to lie on the upper surface of the central spindle, which at first forms at right angles to the polar axis of the egg. Finally the chromatin is drawn into the equator of the spindle, where it forms the equatorial plate. As the poles still continue to separate, the spindle sinks into the egg and rotates through an angle of 90 degrees. After it has reached its maximum growth (Pl. XI, Fig. 19), the second maturation spindle comes to rest at the upper pole of the egg. It is worthy of note, in the egg of *Limax*, that during the forma-

<sup>1</sup> This account of the formation of the rays of the second maturation spindle differs from Mead's account of the formation of the aster in the second maturation spindle in *Chaetopterus*, where the rays of the old aster are merely focussed on new centrosomes.

tion and rotation of the second polar spindle the astral rays are always straight. There is not the slightest indication of a spiral arrangement of the aster, so that mere migration of the spindle cannot explain the spiral twisting of the astral rays that follows later. The centrospheres of the second polar spindle are very similar to the second stage of those already described for the first polar spindle; compare Pl. XI, Figs. 19 and 8, and Figs. 20 and 7. The centrosphere contains a deeply staining center, surrounded by a less deeply staining peripheral zone from which the astral rays diverge; no definite central granule is distinguishable. The centrosphere may enlarge, but it always retains this general character throughout the stage of the second maturation spindle, and never develops the sharply marked zones that are characteristic of the archi-amphiaser stage.

Notwithstanding the similarity that often exists between the first and second maturation spindles, they can always be identified by the presence of peculiar deeply staining bodies that group themselves around the equator of the second spindle. Only one of these bodies is represented in Pl. XI, Fig. 19, but there are many similar structures in the egg. They are more or less regular in outline, being round or slightly oval, but they are apparently without any definite structure. In the earlier stages they are sometimes present as vague bodies lying around the periphery of the egg, but just before the extrusion of the second polar globule they tend to aggregate around the spindle in the upper hemisphere. These bodies probably owe their origin to the circular rings in the ovarian eggs, and are no doubt to be regarded as the yolk-nuclei of various authors.

There is no apparent difference in the size of the two astrosphaeres of the second polar spindle. The distal aster presses close against the periphery of the egg, which again becomes slightly flattened just before the extrusion of the second polar globule. The time required for the formation and separation of the second polar globule is about two minutes—the same as that required for the formation and extrusion of the first. After the second polar globule has been extruded, the egg-nucleus immediately forms a membrane and becomes

vesicular. Although the nucleus increases in size, it retains its position with reference to the place of formation of the polar globules, being frequently held fast to the egg-membrane by a "Zwischenkörper" (Pl. XII, Fig. 27), which often persists until both nuclei have reached their maximum growth. The persistence of the Zwischenkörper is of some importance in the later stages, being often the only means of distinguishing the egg-nucleus from the nucleus of the spermatozoön.

During the formation and growth of the egg-nucleus the astrosphaere undergoes a series of very remarkable changes, which may be referred to three distinct periods. These periods are characterized by the formation, the growth, and the disappearance of the spiral astrosphaere. From the time of the extrusion of the second polar globule until the astrosphaere begins to enlarge, the centrosphere retains unchanged the character of the centrospheres of the second maturation spindle (Pl. XII, Fig. 22). The astral rays are at first straight, but while the aster is still small they begin to bend spirally. When seen from the upper pole of the egg, the rays of the spiral aster are bent toward the right in the direction of the movement of the hands of a clock. Kostanecki and Wierzejski have figured an aster in the egg of *Physa*, which shows the rays at the inner pole of the second maturation spindle arranged spirally; the time of the appearance of the spiral aster in *Physa* corresponds exactly, therefore, to the time of the formation of the spiral aster in *Limax*.

During the second period, the period of growth of the spiral aster, the centrosphere again becomes arranged in distinct zones (Pl. XII, Fig. 25). During this period the center of the aster shows a deeply staining central body, surrounded by a clear zone ("heller Hof"), which is traversed by a loose reticulum. This reticulum connects the central body with the periphery of the sphere from which the astral rays diverge, and seems to be formed by prolongations of the inner ends of the astral rays. Sometimes an extremely minute granule (the "centriole" of Boveri?) can be seen at the center of the homogeneous central body (Pl. XII, Fig. 25). This deeply staining central body is probably homologous to the deeply staining mass of granules



in the middle of the centrosphere of the archiamphiaster (*cf.* Pl. XII, Fig. 25, with Pl. XI, Fig. 3). The rays of the spiral aster continue to lengthen, so that the entire cytoplasm is now involved in the formation of a spiral, which extends from the egg-astrosphaere as a center to the circumference of the egg (Pl. XII, Fig. 22). Pl. XII, Fig. 23, shows a section taken through the spiral aster near the equator of the egg. It also shows that the rays are bent so as to form a right-handed spiral.

The third period is the period of the disappearance of the egg-astrosphaere. The central body, or centrosome (Pl. XII, Fig. 25), gradually fades away (Pl. XII, Figs. 26 and 27) and gives place to a reticulum which traverses the entire sphaere (Pl. XII, Fig. 28). In this stage the centrosphere corresponds exactly to the reticulated centrosphere figured by Wilson for *Toxopneustes*,<sup>1</sup> and by Brauer for *Artemia salina*. During the third period the spiral aster is still plainly visible, but it is less distinct than during the second period. As the nucleus enlarges it gradually encroaches on the reticulated centrosphere, and finally comes to occupy the entire site of the sphaere, so that the astral rays now seem to diverge directly from the periphery of the nucleus as a center (Pl. XII, Fig. 31). The rays become more and more vague, until they disappear and nothing remains of the egg-astrosphaere. The maturation of the ovum is now complete.

## II. STRUCTURE AND MATURATION OF THE SPERMATOZOÖN.

### 1. *Structure of the Spermatozoön.*

The spermatozoa were mounted whole on the slide, and killed in a  $\frac{1}{3}$  per cent solution of acetic acid, and also in a saturated solution of corrosive sublimate to which 5 per cent acetic acid was added. They were then stained with Heidenhain's iron-haematoxylin and with various double stains, such as Lyons blue and borax-carmin, iron-haematoxylin and orange G., acid green and eosin, eosin and haematoxylin. After all of these

<sup>1</sup> Diagram F, Fig. 108, in "The Cell," Wilson.

methods of staining, the spermatozoön shows always a precisely similar structure.

In the last stage of the formation of the spermatozoön of *Limax agrestis* the sperm-head appears heart-shaped in optical section. The tail of the spermatozoön is very long and slender, as is usual among the mollusca. The tail becomes somewhat thickened near the head and passes into the head as an axial rod. In cross-section the axial rod is seen to be surrounded by a mass of chromatin in the shape of a trefoil. The maturation of the spermatozoön seems to be completed by the spiral twisting of the sperm-head; the proximal part of the tail is also involved in the formation of the spiral, as shown in Pl. XII, Fig. 40. In the mature spermatozoön the chromatin appears as a delicate though deeply staining cord, twisted spirally round a refractive, colorless axis. I have not been able to differentiate a middle-piece in the spermatozoön. When the mature spermatozoa are taken from the ducts of the ovo-testis and mounted on a slide, the slender distal part of the tail often wraps itself around the proximal part as a whip around the handle.

## 2. *Fertilization.*

The youngest fertilized ova that could be found in *Limax* were those stored in the lower part of the oviduct. I have succeeded in finding but very few ova at this stage. It is impossible to determine how long the sperm-head has been in the eggs that are taken from the oviduct, for the eggs of *Limax* are not laid immediately after copulation. I have dissected individuals immediately after copulation and have found no ova in the oviduct. Moreover, a careful watch has been kept over slugs that have been seen copulating, but they laid no eggs within the following twenty-four hours. The spermatozoa are, therefore, probably stored for some time after copulation before they are used, and the ova are presumably fertilized soon after leaving the ovo-testis.

Whenever the sperm-head is present in eggs from the oviduct, it appears merely as a deeply stained oval body at the periphery of the egg. There is no evidence of an attraction-cone having



been formed by the penetration of the spermatozoön. In the fertilization of the eggs of some of the mollusks, notably in *Arion*<sup>1</sup> and in *Physa*,<sup>2</sup> the tail follows the head of the spermatozoön into the ovum. When the tail is present in eggs, it is generally easily recognized by its strong affinity for certain stains, a characteristic which may be acquired after its entrance into the egg, according to Van Beneden and Platner. I have never seen any evidence that the tail of the spermatozoön enters the egg of *Limax*, but the difficulty of obtaining eggs at the time of the earliest contact of the spermatozoön with the ovum makes it impossible to determine whether the tail-piece has really penetrated the egg and there broken down, or whether it has been left outside.

I have never been able to detect a middle-piece in the spermatozoön after it has entered the egg, or to recognize a rotation of the sperm-head. Very little change occurs in the sperm-head from the time it first enters the egg until the eggs are deposited, although when the eggs are laid the sperm has probably been in the egg for some hours. All eggs taken from the oviduct and from the albuminous gland were found in individuals that were dissected in the late hours of the evening—from 8 to 10 o'clock P.M. As eggs are rarely deposited during the night, before the early morning hours, it seems probable that at least several hours must elapse between the time of fertilization and the depositing of the eggs.

While the archiamphiaster occupies the middle of the egg, the sperm-head remains quiescent at the periphery, very near its place of entrance at the lower pole. Sometimes the sperm-head is oblong, and sometimes it is slightly constricted in the middle in the form of a dumb-bell, as in Pl. XI, Fig. 4. It is homogeneous, however, and always stains uniformly. As the spindle moves toward the upper pole of the egg, preparatory to the formation of the first polar body, the sperm-head loses its

<sup>1</sup> Platner, "Ueber die Befruchtung von *Arion empiricorum*." *Archiv f. mikr. Anat.*, Bd. xxvii, 1887.

<sup>2</sup> Kostanecki und Wierzejski, "Ueber das Verhalten der sogenannten achromatischen Substanz im befruchteten Ei: Nach Beobachtungen an *Physa fontinalis*." *Archiv f. mikr. Anat.*, Bd. xlvii, 1896.

homogeneous appearance and becomes slightly granular; at the same time it also becomes more or less vesicular, as in Pl. XI, Figs. 7 and 8. The sperm-head sometimes appears as a deeply staining mass of chromatin surrounding a clear central core. A similar appearance of the sperm is often seen when the sperm-heads are cut transversely in the follicles of the ovo-testis. From these appearances, and from the absence of any definite middle-piece to the spermatozoön, and also, as we shall see later, from the usual absence of sperm-asters during the early stages, the suggestion arises that the middle-piece may possibly be surrounded or overgrown, as it were, by the sperm-head, so that the centrosome or centrosomes, if there be any, come to lie within the nucleus. There is, however, no direct proof of this.

During the stage of the first maturation spindle small round bodies are often found accompanying the sperm-nucleus, as in Pl. XI, Fig. 7, and Pl. XII, Fig. 43. They are similar in size to the large yolk-granules, but they stain very deeply like the chromatin. They vary in number; generally there are from two to five of these bodies, but they are always found in the immediate vicinity of the sperm-head. Small deeply staining bodies similar to those in *Limax* are shown occasionally at the periphery of the egg in *Physa*, but no function has been ascribed to them.

Foot has described granular bodies ("sperm-granules") which accompany the sperm-head in *Allolobophora foetida*. In this form the author ascribes their origin to the breaking down of the original sperm-asters. In the fertilized ovum of *Limax* the similar behavior of chromatin and of these tiny bodies toward certain stains suggests that they may owe their origin to particles of chromatin that are constricted off from the sperm-nucleus before it becomes vesicular. I have seen a few cases in which a portion of the chromatin seemed to be in process of constricting from the sperm-head, but such cases are not of very frequent occurrence. After the extrusion of the first polar globule these deeply staining bodies are rarely found in connection with the sperm-nucleus. Still later they disappear, or become scattered through the cytoplasm of the egg, as in Pl. XII,

Fig. 39. What the function of these bodies is I have not been able to determine.

During the stage of the second maturation spindle I have been fortunate enough to find an apparently normal egg in which the sperm is accompanied by two tiny asters. The sperm-head, the sperm-asters, and a part of the second maturation spindle are shown in Pl. XI, Fig. 20, *a*, *b*, *b'*, and *c*. The asters lie some distance away from the sperm, and between the sperm-head and the maturation spindle. These relations of the sperm-head, the asters, and the maturation spindle are precisely similar to those figured by Kostanecki and Wierzejski for *Physa*,<sup>1</sup> and also to those described by Wilson, Boveri, and Hill for the echinoderm, and by Wilson and by Mead for the annelid. During the formation of the second polar spindle the sperm-head becomes more and more vesicular and moves away from the periphery of the egg, in toward the egg-astrosphaere. I have found a single egg in which the centrosome and aster are present, in connection with the sperm-nucleus, immediately after the extrusion of the second polar globule, but the egg is evidently abnormal. Sections through the sperm-nucleus of this egg are shown in Pl. XII, Fig. 21, *a*, *b*, and *b'*. The nucleus is enormously distended and completely enclosed by the rays of a spiral aster, which is formed about a deeply staining granule—the centrosome. There is so little chromatin in the nucleus that it is barely perceptible in the sections. I have repeatedly studied the early maturation stages of *Limax agrestis* in the hope of finding other asters; but, except in the two cases just described, I have found only two or three extremely doubtful cases. These cases, however, are of especial interest, for they serve to throw light on a problem that in *Limax agrestis* is extremely difficult of solution. They show that in all probability, even in these very early stages, structures are present in the cell which do not usually manifest themselves until a much later period, although in most of the forms that have been carefully studied—echinoderms, mollusks, and worms—the sperm-asters appear very soon after the spermatozoon has entered the egg. When the

<sup>1</sup> *Archiv f. mikr. Anat.*, Bd. xlvii, 1896, Pl. XVIII, Fig. 11.

centrosomes first appear in the eggs of *Limax*, they are so extremely minute that it would not be possible to recognize them were it not for the presence of the centrosphere or the astral rays by which they are surrounded. Free from these structures, they would appear only as minute granules (microsomes), from which they are quite indistinguishable.

After the extrusion of the second polar globule the sperm-nucleus begins to grow rapidly. It becomes vesicular, develops a distinct membrane, and rises rapidly through the spirally arranged cytoplasm toward the egg-nucleus, which lies at the upper pole (Pl. XI, Fig. 28). As the egg-nucleus and sperm-nucleus enlarge, they keep pace with each other in development, and by the time the sperm-nucleus has reached the upper pole of the egg both nuclei have attained considerable size. They are often temporarily prevented from coming directly in contact with each other by the enormous reticulated centrosphere, which still persists in connection with the egg-nucleus.

After the centrosphere has disappeared and the two nuclei have attained their maximum growth, as shown in Pl. XII, Fig. 32, they lie side by side at the upper pole of the egg. No difference in size or structure can be detected; they very frequently, though not always, contain even the same number of large nucleoli. There is, however, a constancy in the relative position of the two nuclei, by which they can often be recognized with perfect certainty. The egg-nucleus retains its position at the periphery of the egg, where the *Zwischenkörper*, that was formed between the second polar globule and the egg, still persists. Moreover, the sperm-nucleus, though touching the egg-nucleus, generally lies a little deeper in the egg.

The appearance of the egg of *Limax agrestis* at this stage is precisely the same as that figured by Mark for the egg of *Limax campestris*, with the exception that the nuclear membrane of *Limax agrestis* is always perfectly spherical in outline, never amoeboid, as Mark had shown for *Limax campestris*. It can easily be seen, by a comparison with the living egg, that the even outline of *Limax agrestis* is not the result of swelling caused by the killing fluids, for the nucleus in the living egg



is also perfectly spherical. Up to the time of the apposition of the nuclei no asters are usually present in the eggs of *Limax*, and the centrosomes, even if they be present, cannot be recognized. When the asters first appear, they are almost always in closer connection with the sperm-nucleus than with the egg-nucleus (Pl. XII, Fig. 36), though sometimes they appear directly between the two (Pl. XII, Fig. 33). There is no evidence that a central spindle has been formed between the two centrosomes when both asters are present. Sometimes one aster appears before the other, as Mark has shown in *Limax campestris*. The centrosomes look like homogeneous refractive bodies, which stain very deeply with Heidenhain's haematoxylin. When the asters first appear, there is no differentiation of the center of the aster into a centrosphere. The astral rays are at first few in number and relatively short, but very distinct. As the asters enlarge, the rays increase in number and length, reaching far out into the cytoplasm and sometimes coming into contact with the periphery of the egg. After the formation of the asters, the nuclear membrane is often slightly flattened on the side toward the aster, but the rays do not seem to penetrate the nuclear membrane (Pl. XII, Figs. 34 and 38).

When the asters first appear, the chromatin in the nuclei is in the form of small granules which show no regular arrangement, but are scattered irregularly throughout the nuclei (Pl. XII, Figs. 33 and 34). Later, while the nuclear membrane still persists, the chromatin granules become arranged in rows along the nuclear filaments within the nucleus (Pl. XII, Figs. 35 and 37); outside of the nuclear membrane, between the nucleus and the centrosome, the astral rays appear to be directly continuous with the nuclear filaments, and show distinct varicosities.

As the sperm-asters gradually give rise to the segmentation spindle, and the nuclei come to lie side by side at the equator of the spindle, as shown in Pl. XII, Fig. 38, the sperm-nucleus and the egg-nucleus can no longer be distinguished from each other. The nuclei soon lose their membranes without forming a segmentation nucleus, leaving the chromatin lying at the equator of the spindle in two distinct masses. Later, when

the chromatin is brought into the equatorial plate, the separate origin of the maternal and paternal chromatin is no longer recognizable.

When the astrosphaeres of the segmentation spindle first form, the centrospheres show no differentiation into zones such as has been described for the centrospheres of the maturation spindles. After the spindle has fully formed, however, the "cortical" and "medullary" zones begin to develop around the centrosome, so that the astrosphaeres that appear in connection with the sperm-nucleus are generally structures similar to those of the maturation spindle.

When the segmentation spindle is completely formed and the egg is ready to divide, the spindle lies transverse to the polar axis in the upper hemisphere in a mass of protoplasm that stains more deeply than the rest of the egg. After the maturation spindle has divided, and the two resulting daughter-nuclei have come to rest, all trace of the centrosomes and asters is lost. When the daughter-cells are again ready to divide, the centrosomes and asters reappear on the periphery of the nucleus, and gradually separate to form the segmentation spindle of the next division, and so on.

### III. OBSERVATIONS ON ABNORMAL OVA.

#### 1. *Polyspermy.*

I hoped to find in polyspermic eggs a clue to the origin of the sperm-asters. Polyspermy rarely occurs in *Limax*, however, and when cases of it are found they throw no light on the origin of the centrosomes. I have found single capsules containing as many as twenty-five eggs, some of which were unsegmented and apparently normal; others had divided into two cells; still others into four cells. The unsegmented eggs when killed in Vom Rath's fluid and mounted whole, unstained, showed as many as three small vesicular sperm-nuclei in addition to the usual large egg-nucleus. One set of abnormal eggs suspected of being polyspermic, when sectioned, showed that in some cases as many as twelve spermatozoa had entered a



single egg. Most of the sperm-nuclei had become vesicular and had migrated toward the egg-nucleus at the upper pole. These eggs were very irregular in outline, and the cytoplasm showed that degeneration had already begun. None of these polyspermic eggs showed any evidence of division, *and in none of them was there any evidence of the presence of centrosomes or asters.* I have occasionally found isolated cases of polyspermy among normal sets of eggs, but except for the presence of more than two nuclei, they gave no evidence of being abnormal. Polyspermic eggs are evidently incapable of development in *Limax agrestis*; normal development ceasing before the sperm-nuclei attain their full growth and, therefore, before the asters appear.

## 2. Abnormalities Other than Polyspermy.

A few abnormal eggs showed very peculiar bodies in connection with the sperm-nucleus. I have seen a number of eggs at the stage when the asters usually appear, in which the sperm-nucleus is accompanied by two relatively large spherical bodies, which lie out in the cytoplasm on each side of the sperm-nucleus. These bodies are on the side of the sperm-nucleus away from the egg-nucleus, but from their number and evident connection with the sperm-nucleus one can scarcely avoid the conjecture that they bear some relation to the centrosomes. I have been much puzzled by these bodies and thought that I might be dealing with two species of *Limax*, in one of which the centrosomes appeared as naked sphaeres. I am indebted to Prof. H. A. Pilsbry, of the Philadelphia Academy of Natural Sciences, for having identified all the individuals as belonging to the single species, *Limax agrestis* (Linné).

In two instances at least, the eggs in which these structures occur are known to have been abnormal, for the apposition of the egg and sperm-nuclei did not occur until five hours had elapsed after the eggs were laid. Normally this stage was reached within two hours.

Before the extrusion of the second polar globule the upper poles of the eggs became very irregular in outline and formed pseudopod-like projections from the upper surface. After the

extrusion of the second polar globule the eggs regained their normal outline. In some of these abnormal eggs the egg-nucleus and sperm-nucleus remained permanently separated, although both had attained their maximum size.

The sperm-nucleus is sometimes held fast to the egg-membrane at the lower pole, or it may have penetrated only half-way into the egg. While the sperm-nucleus is thus retarded in its advance toward the egg-nucleus, the two spherical bodies appear. Pl. XII, Figs. 41 and 42, show two sections through different regions of the same egg. The section in Pl. XII, Fig. 41, which is the last of a series, falls through the sperm-nucleus on the side away from the egg-nucleus, and shows the sperm accompanied by the two distinct bodies. These bodies behave differently toward different staining reagents. This egg was killed in corrosive sublimate acetic acid (5 per cent) and hardened in Flemming's solution. When stained in Heidenhain's haematoxylin, the bodies appear as deeply stained homogeneous structures with well-defined outlines; but in borax-carmin and Lyons blue they are highly refractive and almost colorless. Around one of these bodies is a slightly denser layer of protoplasm, which bears out the suggestion that they may be centers of attraction. There is, however, no radiate structure around either of them. Nevertheless, their late appearance, their connection with the sperm-nucleus, their behavior towards staining reagents, their number and general structure make the suggestion irresistible that they may be due to the presence of the centrosomes which have taken this peculiar form under abnormal conditions. If these conclusions be well founded, these abnormal ova furnish important evidence in regard to the nature of the centrosome in *Limax*, for they show that centrosomes may exist as definite structures in the egg apart from the rays which usually mark their presence.

#### IV. FORMATION OF THE SPINDLE.

Soon after the apposition of the egg and sperm-nuclei the chromatin becomes arranged along the nuclear filaments that lie in the long axis of the forming spindle (Pl. XII, Figs. 36

and 37). This process takes place before the breaking down of the nuclear membranes. The intimate connection between the chromatin and the nuclear filaments before the loss of the nuclear membrane seems to support the view held by Flemming, Reinke, and Wilson, that the chromatin is in contact with the mantle fibers from the beginning, and not secondarily brought into connection with them by the ingrowth into the nucleus of the polar rays. If, however, the spindle is formed entirely by a morphological rearrangement of the nuclear filaments that are focussed at the centrosome, it is not wholly clear why the nuclear membrane on the side toward the aster should be flattened as if by pressure exerted on the membrane by the astral rays. Moreover, the explanation of the formation of the spindle by a rearrangement of nuclear substance does not account for the formation of the second maturation spindle of *Limax agrestis*. The second maturation spindle is formed *within the centrosphere* (Pl. XI, Fig. 16), while the old astral rays still persist. The new asters are formed by rays that reach through the centrosphere out into the cytoplasm, so that immediately surrounding the centrosphere two sets of radiating fibers can be detected — those that belong to the central astrosphaere of the first maturation spindle, and those that belong to the asters of the second maturation spindle in process of formation.

It is not possible to explain the formation of the second maturation spindle on any theory of a mere rearrangement of a preëxisting structure in Wilson's sense. The process seems to be exactly the reverse of focussing; for the rays are projected from the centrosome out into the centrosphere, which appears almost structureless. Meanwhile the centrosphere in which the spindle lies is constantly expanding and acquiring an ever increasing diameter until it finally fades out. The astral rays seem to be formed *de novo* about the centrosome; hence Wilson's explanation of the organic growth of the astral rays may apply not only to their continued growth, but also to their origin. While the spindle is forming, the chromatin still lies outside the sphaere, on the upper surface, where it was left after the extrusion of the first polar body (Pl. XI, Fig. 15). Later the chromatin is drawn into the equator of the spindle,

but not until after the spindle has already formed. Hence, in the formation of the second maturation spindle, only the mantle fibers of the spindle *could* be formed from nuclear substance, as Flemming and Reinke maintain.

I am unable to determine the precise method by which the chromatin is brought into the equator of the second polar spindle. It seems, however, as if it must be effected by some of the polar rays that come secondarily into contact with the chromatin, and thus become transformed into mantle fibers.

#### V. STRUCTURE OF THE CYTOPLASM: ARCHOPLASM.

The very coarse cytoplasmic network that is often seen in the egg of *Limax* is probably not characteristic of the living egg. A coarse reticulated appearance is nearly always found in eggs that have been preserved in corrosive sublimate acetic solution, and is in all probability due, in part, to precipitations that are formed when the eggs are killed. This structure of the cytoplasm occurs in eggs that are apparently perfectly preserved and that show the finest details of structure in the astrosphaeres (Pl. XI, Figs. 2, 5, and 10). Probably a truer representation of the structure of the cytoplasm is seen in those eggs that were hardened in Flemming's solution after they had been killed in corrosive sublimate acetic solution. These eggs show no distinct reticular structure, but minute varicosities are distinguishable on threads of the utmost fineness. The general appearance of such a preparation is granular rather than reticular, although the filamentous nature of the cytoplasm in the preserved material is still faintly discernible. In these eggs the astral rays are extremely delicate, and the details of the centrosphere are clearly shown.

The peculiar structure of the centrosphere, particularly at the stage of the archiamphiaster, cannot be due to the method of preservation of the eggs, for it is constantly present, no matter with what reagent the eggs have been killed. Preparations have been made of eggs that were killed in Flemming's solutions, in corrosive sublimate acetic (5 per cent), in chromic acid (1 per cent), and in Flemming's solution after corrosive











sublimate acetic (5 per cent), and also in picro-acetic; all these agree in showing similar structures at corresponding periods.

The archiamphiaster in the egg of *Limax* is of particular interest, as the centrosphere corresponds very closely to the type of centrosphere in which Boveri first described the "archoplasm" in the eggs of *Ascaris*. In *Limax* the outer zone of the centrosphere, which corresponds to the "archoplasmic" zone of Boveri, appears as a deeply staining homogeneous ("cortical") zone surrounding a clear ("medullary") zone which is not stained by any of the methods used in coloring the preparations. The contrast between the "cortical" zone of the centrosphere, or the "archoplasm," and the cytoplasm is sharpest in those eggs that have been killed and hardened in their capsules in Flemming's solution. This shows that the appearance of the "archoplasm" is sometimes modified by the killing reagent. It is not, however, produced by it.

When the sections are treated with the double stain, Lyons blue and borax-carmin, the centrosome and "cortical" zone ("archoplasm") of the centrosphere both stain blue, while the spindle, the astral rays, and the cytoplasmic network take the red stain of the carmin. After the first polar globule has been extruded and the egg-centrosphere has become uniformly granular in appearance, as shown by Pl. XI, Fig. 14, the whole centrosphere takes a faint bluish stain after Lyons blue and borax-carmin. The second maturation spindle is formed within the blue staining centrosphere, and if Boveri's theory of archoplasm holds, the second maturation spindle should stain blue. This is not the case, however. *The astral rays of the second maturation spindle are red like the cytoplasm, even while the spindle lies within the centrosphere.* The centrosomes of the archiamphiaster appear faintly bluish, but even the tendency of the centrosomes to take the blue stain constantly diminishes. At this stage the Zwischenkörper of the first polar globule also stains blue, like the archoplasmic zone in the archiamphiaster stage.

After the extrusion of the second polar globule the distinction between those structures in the astrosphaere that stained blue and those that stained red in the earlier stage is still

more indistinct, until finally the color distinction between different structures is wholly lost. During the stage of the archiamphaster the chromatin of both the egg-nucleus and the sperm-nucleus stains *blue* after corrosive sublimate acetic (5 per cent). When the nuclei have attained their maximum growth the chromatin stains *red* after corrosive sublimate acetic (5 per cent).

The only generalization that seems justifiable from the color reactions in the egg of *Limax* is that the different structures in the cell periodically undergo chemical changes in their constitution. Indeed, there is some evidence from the color reactions that these changes in color may correspond to phases of a cycle through which the protoplasm of the cell passes during development. There is, however, no evidence for the existence of an "archoplasm" which is distinct from the general cytoplasmic reticulum.

It has been assumed in the case of *Ascaris* that the cleavage stages follow one another in such rapid succession "that the character of the centrosphere has not time to change." According to Boveri's account of *Ascaris*, "Schon während der Bildung des Richtungskörpers finden wir das Archoplasma wenn auch weniger verdichtet und nach aussen allmählich sich verlierend um das Spermatozoon angehäuft; noch früher dagegen lässt sich seine Existenz nicht nachweisen."

In *Limax*, on the contrary, the peculiar type of centrosphere that is characteristic of *Ascaris* is found in ova only during a long period of quiescence, lasting from the time their development in the ovary is completed until the formation of the first polar globule. After that the centrosphere never again acquires such a high degree of differentiation, although, as we have seen, it still undergoes a series of changes that are repeated during the maturation and fertilization stages of different ova with the greatest precision. Kostanecki has attempted to explain the different phases under which the "archoplasm" of *Physa* appears as due to various changes that occur in the structure of the cell in the course of development. These changes consist in varying relations between the yolk, the vacuoles, and the protoplasm, and they show that the formation

of the "archoplasm" in *Physa* depends merely on the way in which the protoplasm is collected around the centrosome.

The cytoplasmic nature of the "archoplasm" in *Limax* is revealed by various changes that go on in the centrosphere itself, rather than by changes in the surrounding cytoplasm. Pl. XI, Figs. 10 and 13, and Pl. XII, Figs. 22, 25, 27, 28, and 30, show successive stages in the disappearance of the "archoplasm" and of the "centrosome" of the *Ascaris* type. Pl. XI, Fig. 13, shows the centrosphere approaching the type of the "attraction sphaere" of Van Beneden, or the "Mikrosphaere" of Heidenhain. The outer "cortical" zone of the centrosphere is bounded by a circle of granules (microsomes) in which the radially arranged cytoplasmic fibers at first seem to terminate. Within the centrosphere the astral rays are continued as extremely delicate fibers which traverse the "cortical" zone and terminate on the periphery of the "medullary" zone. The central zone is uniformly finely granular in appearance. It is without a distinct center and is not penetrated by the radial fibers. In the second stage of growth of the centrosphere, after the extrusion of the second polar globule, there is no trace of a distinct homogeneous ("cortical") zone. The astral rays come closer together as they approach the periphery of the medullary zone or "heller Hof" (centrosome of Boveri), but they always remain distinctly separate. They can be traced into the reticulum which traverses the "heller Hof" and which is attached to the centrosome at the center of the sphaere. The "cortical" zone of the archiamphiaster stage now corresponds to that part of the cytoplasmic reticulum which immediately surrounds the "heller Hof," and which, by the radial arrangement of its fibers, forms the central rays of the aster.

Moreover, the transformation of the aster into a spiral that involves the cytoplasm of the entire cell after the extrusion of the second polar globule leaves little doubt as to the cytoplasmic nature of the aster. As the spiral rays diverge from the centrosphere, they gradually break up into the cytoplasmic reticulum. During the period of the disappearance of the egg-centrosphere the sphaere is still further resolved into the

structure of the cytoplasm, and there is a complete disappearance of any specialized central body. At this stage the centrosphere corresponds to the type of the "reticulated" centrosphere that Wilson has described for *Toxopneustes*, and that Brauer has figured for *Artemia*.

The series of successive stages through which the centrosphere of *Limax* passes seems to show that a highly specialized astrosphaere can be resolved into the reticulum of the cytoplasm by a gradual relaxation, as it were, of a tension exerted on the surrounding contents of the cell from a focal point, the centrosome. These observations on the eggs of *Limax* are in accordance with the view held by Van Beneden, Heidenhain, Reinke, Wilson, Kostanecki, and others, that the "archoplasm" has no existence as a specific substance, but is only a part of the general cytoplasm.

## VI. THE CENTROSOME.

The study of the ova and the spermatozoa of *Limax agrestis* throws little light on the origin and nature of the centrosome. In the ovum the centrosome appears under widely different forms in different stages. First, it is seen as a small deeply staining point in the middle of the aster, as in Pl. XI, Fig. 1. In the archiamphiaster stage, after a long period of growth, the central body or centrosome is composed of a mass of granules (Pl. XI, Figs. 2 and 3). After the centrosome has divided in this stage the two resulting centrosomes appear as dumb-bell-shaped rods (Pl. XI, Figs. 2 and 4). After the extrusion of the first polar globule the centrosome again appears as a single tiny granule that stains very deeply with Heidenhain's haematoxylin (Pl. XI, Fig. 14). After the extrusion of the second polar globule the central body apparently corresponds to the granular centrosome of the archiamphiaster stage; it reaches an enormous size (Pl. XII, Fig. 25), after which it becomes resolved into a reticulum and finally disappears (Pl. XII, Figs. 27 and 30).

The centrosomes of the segmenting egg appear in connection with the sperm-nucleus, though I have never been able to



trace their origin to a middle-piece in the spermatozoön. The time of their appearance is variable, but they usually are not visible, even if present, until the egg and sperm-nucleus have reached their maximum size. There is no reason to believe that asters that appear during the early fertilization stages in the egg of *Limax* are temporarily lost only to appear later, just before the formation of the segmentation spindle, as in *Allolobophora foetida*. The asters once formed in connection with the sperm-nucleus persist, but only in very exceptional cases are they formed at all before the apposition of the egg and sperm-nucleus. Crampton has recently described a somewhat similar case in the egg of an Opisthobranch, *Bulla*, in which the centrosomes do not appear in the fertilized egg until the time of apposition of the pronuclei.

## VII. SUMMARY.

The centrosome in the egg of *Limax agrestis* appears under different forms during the maturation of the egg. Sometimes the centrosome appears as a group of granules, as in the archiamphiasier stage. The granular centrosome at each pole of the archiamphiasier is often divided into two distinct groups or centrosomes, each of which is in turn composed of granules that seem to be connected with each other, and appear dumb-bell-shaped. Sometimes the centrosomes appear as single granules, as in the centrosomes of the second maturation spindle. The centrosome is seen as a large spherical body after the extrusion of the second polar globule. The centrosome then becomes granular and finally is resolved into a reticulum without dividing, after which no definite centrosome can be detected.

The character of the entire centrosphere changes during the maturation stages. First, the centrosphere appears as a series of concentric zones; after this as a deeply staining center with a single limiting zone; then as a reticulated sphaere with a large homogeneous body in the middle; and finally as a reticulated sphaere. The egg-centrosphere then disappears. The various zones of the centrosphere are formed from the cytoplasmic reticulum of the egg.

There is no evidence of an "archoplasm" which is distinct from the cytoplasm of the egg in *Limax*.

There is no middle-piece to the spermatozoön. The asters of the segmentation spindle very rarely appear before the apposition of the egg-nucleus and the sperm-nucleus. Sometimes, though very exceptionally, they appear before the maturation stages of the egg are completed. The sperm-asters cannot be traced directly to the spermatozoön, although they are more closely connected with the sperm than with the egg-nucleus. The nuclei do not unite to form a segmentation nucleus.

The spindle in *Limax* is not formed wholly by a rearrangement of nuclear substance.

While the old astral rays still persist around the centrosphere of the first maturation spindle, the second maturation spindle is formed *within* the centrosphere. The polar rays of the second maturation spindle project through the centrosphere out into the cytoplasm; they are not formed by the focussing about new centers of rays that are already formed.

The asters of the second maturation spindle are newly formed structures. The mantle fibers of the second maturation spindle seem to be derived secondarily from polar fibers. The formation of the spiral aster in *Limax agrestis* occurs soon after the extrusion of the second polar globule, and only then. When seen from the upper pole of the egg the rays of the spiral aster are bent in the direction of the movement of the hands of a clock.

The spiral aster is derived secondarily from an aster whose rays are at first straight. Normally, the spiral arrangement of the astral rays occurs in connection with the egg-aster. It may, however, occur in connection with the sperm-aster under special conditions.

## VIII. APPENDIX.

### *Material: Collecting and Keeping.*

*Limax agrestis*, Linné, is a species of *Limax* common in Europe. It has been introduced into this country and has become very abundant in the neighborhood of Philadelphia.

During the early fall months I collected large numbers of *Limax* in old vegetable gardens in Bryn Mawr and under stones that lay along the banks of an open drain. These slugs can be collected out of doors as late as the middle of December. Much of my material was collected late in the evening, with the aid of a lantern, and in the early morning hours when the slugs were on their way to their hiding places. During the winter I was able to collect *Limax* in small numbers in the carnation beds of a neighboring hothouse.

The slugs were kept in the laboratory in a large Wardian case filled with living plants, and were fed on cabbage leaves, plantain, dock, and various vegetable roots. Under these conditions the slugs lived for some time in an apparently healthy condition and yielded eggs in abundance. Sooner or later, however, slugs that are kept in confinement become infested with parasites, even when the greatest precautions are taken as to cleanliness and an abundant food supply. The ovo-testis becomes infested with parasitic protozoa, which are sometimes found in swarms in the capsules after the eggs have been laid. A parasitic thread worm is also found in the reproductive organs of *Limax*.

Eggs enclosed in capsules that contain even a great many parasites are by no means necessarily abnormal in their development; indeed, they often give rise to normal embryos. In order to avoid any complication that might result from the study of abnormal eggs, however, fresh relays of slugs were constantly supplied from time to time in place of the old ones.

#### *Preparation of Material.*

The egg of *Limax* is imbedded in an almost liquid jelly contained in a large, tough capsule. The egg is immediately surrounded by a somewhat denser layer of jelly, which adheres very closely to its surface.

At first I attempted to preserve eggs in the capsules, and for this purpose Flemming's solution and chromic acid (1 per cent) were chiefly used. The killing fluids quickly penetrated the capsules so that the eggs were perfectly preserved. The cap-

sules containing the eggs were then washed in water and were afterwards passed through the various alcohols. Preparatory to imbedding the egg in paraffine, most of the capsule was cut away, only that part being left which immediately surrounds the egg. After using this method of preparation it was found to be almost impossible to section the eggs, owing to the extreme brittleness of the hardened jelly around them, so that although the eggs themselves seemed well preserved, the method had to be abandoned as impracticable. It was found necessary to resort to the tedious method of removing the eggs one at a time from the capsules. This was done as follows: Each egg was watched under the microscope until it had reached the desired stage of development; the capsule was then quickly placed in a saturated solution of corrosive sublimate, to which 5 per cent glacial acetic acid had been added. As soon as the egg had become white and opaque, the capsule was removed to a bath of distilled water. The capsules were then opened under water, and the eggs were returned, free from the jelly, to the corrosive sublimate acetic solution, where they were allowed to remain for a few minutes. They were then passed through the alcohols (35, 50, and 70 per cent successively), and finally into 90 per cent alcohol, where they were kept until used.

By far the best results, however, were obtained from eggs that were fixed for from 15 to 20 minutes in Flemming's weak solution, after they had been killed in corrosive sublimate acetic solution. This method is always [uniformly] successful, and the preparations made by it show the minutest details of structure.

A few good preparations were also obtained by the use of picro-acetic acid, 2 per cent, as a fixative after corrosive sublimate acetic. This method was, however, generally unsatisfactory, as the picric acid often completely destroyed the structure of the centrosphere. The great advantage of corrosive sublimate acetic over other killing reagents is that it penetrates the capsule very quickly without hardening it. If the corrosive sublimate acetic solution be allowed to act too long on the capsules, so that the inner layers become toughened,



the difficulty of removing the egg from the jelly is greatly increased. The jelly can still be removed, however, by allowing the egg to stand in water, but this method should be avoided, as it is apt to impair the structure of the egg. Picric acid, chromic acid, and Flemming's solution toughen the liquid contents of the capsule so rapidly, that after using them it is often extremely difficult to free the eggs from the capsules without injuring them.<sup>1</sup> For this reason these solutions were avoided as killing reagents.

On account of the small size of the eggs it was found expedient to stain them before imbedding in paraffine. The eggs were imbedded in hard paraffine (56 per cent) and cut 3 and 4  $\mu$  thick. Each egg gave from 20 to 25 sections. These were mounted serially with Mayer's albumen and water, care being taken to wash off as much of the albumen as possible. The sections were afterwards stained on the slide in Heidenhain's and Delafield's haematoxylin after iron-alum.

Various combination stains were also used, chiefly Korscheldt's double stain, Lyons blue and borax-carmin, and iron-haematoxylin with orange G.

BRYN MAWR COLLEGE, June, 1897.

<sup>1</sup> In place of needles, fine porcupine quills were used in opening the capsules. They proved most efficient implements for the purpose, and have the added advantage that they are not acted upon by corrosive sublimate and acids.

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## EXPLANATION OF PLATE XI.

*All figures drawn with camera and lenses of Zeiss. Homogeneous immersion, 1.5 mm. apochromatic compensation, ocular 4.*

FIG. 1. Section of ovarian egg containing small astrosphaere.

FIG. 2. Section of ovarian egg containing a well-formed archiamphaster. The centrosphere of the aster consists of two zones, a dark "cortical" zone and a light "medullary" zone. The "medullary" zone contains two oblong centrosomes composed of granules.

FIG. 3. Section of egg just deposited. The egg contains an archiamphaster similar to that in the ovarian egg. In the "cortical" zone to the left of the spindle the central granules are arranged irregularly. In the "cortical" zone to the right the central granules are arranged in two groups.

FIG. 4. Section through one pole of the archiamphaster. The centrosphere consists of four concentric rings. The central granules are grouped so as to form two dumb-bell-shaped centrosomes. The sperm-head is seen at the lower pole.

FIG. 5. Section through pole of archiamphaster. No centrosomes are present.

FIG. 6. Centrosphere and centrosomes of archiamphaster.

FIG. 7. First maturation spindle. Sperm with deeply staining body at lower pole of egg.

FIG. 8. Formation of first polar body. The central centrosphere is enlarging before the separation of the polar body. Sperm-head vesicular.

FIG. 9. Extrusion of first polar body. Centrospheres in the archiamphaster stage.

FIG. 10. Section through egg-astrosphaere after extrusion of first polar body. The centrosphere is beginning to enlarge. The centrosomes are single granules which are beginning to separate.

FIG. 11. Section through egg-astrosphaere after extrusion of first polar body. Beginning of the disappearance of the "cortical" and "medullary" zones.

FIGS. 12 and 13. Later stages than Fig. 11.

FIG. 14 *a, b, c*. Three successive stages through the centrosphere just before the second maturation spindle begins to form.

FIG. 15. Early stage in the formation of the second maturation spindle within the centrosphere. The astral rays still persist. The chromatin lies on the periphery of the sphere where it was left after the extrusion of the first polar body.

FIG. 16. A later stage in the formation of the second maturation spindle. The spindle still lies within the centrosphere, which is outlined by granular thickenings in which the rays of the old aster terminate.

FIG. 17. The second maturation spindle after the disappearance of the centrosphere. Chromatin on the upper surface of the spindle.

FIG. 18. Later stage than Fig. 17.

FIG. 19. Second maturation spindle.

FIG. 20 *a*. Section through pole of second maturation spindle.

FIG. 20 *b* and *b'*. Sperm-asters in the same egg.

FIG. 20 *c*. Sperm accompanying sperm-asters. (Fig. 20 *b* and *b'*)

















## EXPLANATION OF PLATE XII.

*All figures drawn with camera and lenses of Zeiss. Homogeneous immersion, 1.5 mm. apochromatic compensation, ocular 4.  $\times$  667.*

FIG. 21 *a*. Egg-astrosphaere after extrusion of second polar body. The astral rays are straight.

FIG. 21 *b* and *c*. Two successive sections through the sperm-nucleus, which is surrounded by rays of a spiral aster.

FIG. 22. Egg-nucleus and spiral-aster after extrusion of second polar-globule. The centrosphere consists of a deeply staining center surrounded by a light peripheral zone.

FIG. 23. Section through equator of egg containing spiral-aster. Section seen from upper pole. Astral rays bent to right.

FIG. 24 *a*. Section through egg-nucleus and part of astrosphaere.

FIG. 24 *b*. Second polar globule and "Zwischenkörper" of Fig. 24 *a*.

FIG. 25. Centrosphere and astral rays of Fig. 24 *a*. Homogeneous body in center of clear zone which is traversed by a reticulum. The reticulum is formed from inner ends of astral rays.

FIGS. 26 and 27. Sections through egg-centrosphere showing successive stages in the breaking down of the central body.

FIG. 28. Section through the reticulated egg-centrosphere after the disappearance of the central body. The sperm-nucleus and the egg-nucleus are the same size. Egg-nucleus at the upper pole.

FIGS. 29 and 30. Successive stages in the disappearance of the egg-astrosphaere.

FIGS. 31 and 32. Sections through the egg- and sperm-nucleus. The egg-nucleus lies nearer the upper pole of the egg. Fig. 31 still shows indications of the astral rays.

FIGS. 33 and 34. First appearance of the sperm-asters after the nuclei have reached their maximum size and have come into contact with each other.

FIG. 35. Radial arrangement of chromatin within the nuclear membrane.

FIGS. 36 and 37. Section showing closer contact of asters with sperm-nucleus than with egg-nucleus. Formation of segmentation spindle.

FIG. 38. Formation of segmentation spindle between egg- and sperm-nuclei.

FIG. 39. Deep-staining granule (chromatin?) in the cytoplasm near the nucleus.

FIG. 40. Mature spermatozoön.

FIGS. 41 and 42. Sections through an abnormal egg in which the apposition of the egg- and sperm-nuclei occurred five hours after the eggs were laid.

FIG. 41. Section through periphery of sperm-nucleus on side away from egg-nucleus. The sperm-nucleus is accompanied by two refractive bodies (centrosomes?).

FIG. 42. Section through egg- and sperm-nuclei. Egg-nucleus at upper pole of egg near the second polar globule.

FIG. 43. Sperm with deeply staining bodies at periphery of egg.







# LARVAL STAGES OF SCHLOENBACHIA.

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## INTRODUCTION.

*Law of acceleration of development.*—A few years ago naturalists were very much given to speculating about the theory of evolution, reasoning abstractly for or against it, and construct-

ing imaginary family trees. To-day a serious naturalist would just as soon think of taking up cudgels in favor of the theory of gravitation as of evolution. Speculation is no longer popular, and now we wish to know, not whether certain organisms developed out of others, but *how*. This change has largely been brought about by the application of the law of acceleration of development to the study of biology. From the studies of Louis Agassiz and his followers we know that, theoretically, each organism in its ontogeny ought to go through stages of growth corresponding to all its ancestors, and that these stages ought to appear in the order of its ancestral forms. A part of this may be verified in biological laboratories, by studying embryonic and larval stages in animals. Even this is difficult, because the habits of larvae are so different at successive periods of growth that, in confinement, it is often impossible to trace them with certainty through the various stages, and we usually have to take different individuals to show the successive development. And when we attempt to correlate growth stages with ancestral genera the task becomes still more difficult, for then we must leave the living organisms, and are thrown back on paleontology. We often find in a growth stage an association of characters that never occurred in any ancestral form, thus obscuring the parallelism. Then, too, the geologic record is notoriously incomplete, and from the nature of things must always remain so; thus the paleontologic record is often lacking just where we most need it.

#### PALEONTOGENY.

In order to make a satisfactory comparison of ontogeny and phylogeny, the naturalist must select some group in which living and fossil forms are classified on the same basis; such groups are the brachiopods and the molluscs. But brachiopods are scarce, hard to obtain by dredging, hard to rear in marine laboratories, and most of the families are long since extinct, so that ontogenic studies of living species have not thrown much light on the history of the race. Beecher, Schuchert, and J. M. Clarke have, however, succeeded in working out the ontogeny

of a number of fossil species, and have compared the growth stages of these with the history of the group. Here, again, comes in the difficulty that ontogenic series can be obtained only by putting together in a row a number of distinct individuals of various sizes, with the chance of making mistakes in identification increasing as the specimens grow smaller and have fewer characteristic marks. Very naturally, closely related species become more alike as we go down to the younger stages, until it is impossible to tell which species the very young larvae belong to. Of course some species have specific characters thrown back by acceleration until even the earliest larval stages are recognizable, but usually specific characters do not appear until the adolescent period is well advanced. This is true of all marine invertebrates that go through a larval period.

Of living molluscs the gastropods and the pelecypods offer the same difficulties as the brachiopods, and have been much less studied; even of the common oyster not all the larval stages are known, and no other mollusc has been so closely studied as that has.

*Life history of cephalopods.*—The chambered cephalopods offer the best means of comparing ontogeny with phylogeny, although the one available living form, *Nautilus*, belongs to the old unspecialized group of nautiloids that has changed little since its origin. Here, again, we are thrown back on paleontology, but this time the difficulties are not so great, for there is a great group of cephalopods, the ammonoids, that has left in the stratified rocks abundant materials for study.

The ammonoids branched off from the nautiloids in the Upper Silurian or the Lower Devonian, at first small, simple, and rare, but they developed rapidly, until by the end of the Devonian all the groups of goniatites were already present. These increased steadily in numbers, size, and complexity, and during the Carboniferous gave rise to the first simple ammonites; these latter are a distinctly, although not exclusively, Mesozoic race, which developed with wonderful rapidity from the first rare members into numerous families, hundreds of genera, and thousands of species, reaching their acme in the Jurassic. In the Cretaceous they gradually declined, dropping

off one at a time, until all were gone before the end of that age. Only the simple radicles or stocks persisted, but from time to time certain genera branched off from the main line, became highly specialized, and often gave rise to so-called abnormal forms, such as *Hamites*, *Baculites*, *Crioceras*, *Scaphites*, phylogerontic or degenerate genera (retrogressive), which did not perpetuate their race, but soon died out without descendants.

Of course there were many other phylogerontic genera that were not abnormal in form; thus *Clymenia* branched off in the Upper Devonian into a variety of species, and disappeared as suddenly; *Medlicottia* reached its culmination in the Permian, barely managed to live on into the Trias, and disappeared without posterity; while the main stock of unspecialized *Prolecanitidae* endured as long as the race.

In the beginning the number of phylogerontic forms was small, for most of the goniatites left descendants among the ammonites; but their number increased during the Mesozoic, showing a constantly growing tendency to become abnormal, until before the end of the Cretaceous the entire stock of ammonites had become phylogerontic, and died out finally from sheer lack of plasticity to modify itself further with changing conditions.

The life history of the ammonites is a finished chapter in biology, and we have in museums and monographs a nearly complete record of their development. It only remains to study genetic series of ammonites. One way (that usually adopted) is to compare a series of adults from successive geologic periods, and by tracing resemblances to construct theoretical family trees. Results of this work may be seen in text-books of paleontology, and its unreliability may be realized if one ever tries to use these tentative genealogies. This would undoubtedly be the safer way if we had a complete geologic record and if the faunas of the various geographic provinces had been preserved. But since this is not the case, we have to meet conditions as they are, not as they might be.

*Palingenesis*.—The other way is to study the ontogeny of representative species under each genus, and by comparing each stage of growth with antecedent forms to find out the











probable relationship of these genera and the meaning of the growth stages. From the researches of Hyatt, Branco, and Karpinsky we have learned that the ammonoids preserve in each individual a complete record of their larval and adolescent history, the protoconch and early chambers being enveloped and protected by the later coils of the shell. Thus by breaking off the outer chambers successively the naturalist can, in effect, cause the shell to repeat its life history in inverse order, for the ontogeny of the individual is an epitome of the history of the race, and each stage of growth represents, if not always an ancestral genus, at least some of the salient characters of that genus, although unequal acceleration often crowds together in an ontogenic stage characters that occurred in genera widely separated in time. But where the parallelism is at all exact these genera appeared (although not necessarily disappeared) in the order of their minute imitations in the larval history of their descendants; thus by comparing larval stages with antecedent adult forms the naturalist finds the key to relationships and is enabled to arrange genera in genetic series.

The ammonoids were all marine, never parasitic, never fixed in station, and with them no resorption of the shell has ever been noted; thus with them, while there often is some slight obscuring of the record, due to unequal acceleration of certain characters, there is no "falsification of the record." Ancestral characters may not be repeated in the same association in the history of the descendants, but they occur in the same order in which they occurred in the history of the race. So far as the writer's experience goes, these characters shown in the larval stages of ammonites are mainly palingenetic; it is a mistake to give the name of *coenogenesis* to crowding together by unequal acceleration in the descendant of characters that occurred in separate generations of ancestors.

*Omission of stages.*—The only cases known to the writer where stages of growth are actually omitted entirely are: (1) by pushing back remote ancestral stages or characters beyond the protoconch, where they are either lost entirely out of the ontogeny, or at least leave no record in the shell; (2) between the protoconch and the first larval stage. The protoconch is remark-

ably constant in all ammonites, but even in nearly related species of the same genus the first larval stages may be quite different, although the later larval stages may be very similar. It is a well-known fact that free larvae have a much better chance of repeating their ancestral history in unabbreviated form than embryos that go through their development in the egg.<sup>1</sup> It is quite probable that different species of ammonites, just as is the case with living molluscs, were hatched at different stages of growth, and that the omitted stages may correspond to a longer period spent in the egg by one species than that spent by a species that did not omit these stages. Thus certain species of *Schloenbachia* reach the glyphioceran stage immediately after the protoconch, while others go through several generic stages between the protoconch and the glyphioceran stage; this happens in species in the same geologic horizon, so it cannot be due to difference in removal from the parent radicle. Such cases as these make it hard to interpret ontogeny, but they are not "falsifications of the record." Holzapfel<sup>2</sup> has described the young stages of *Anarcestes karpinskyi* Holzapfel, showing that it goes through a typical mimoceran stage, in which the nepionic shell does not touch the protoconch for half a revolution. But in the ontogeny of a nearly related species, *Anarcestes plebeiformis* Hall, as described by J. M. Clarke,<sup>3</sup> this mimoceran stage is omitted, for the shell is close coiled from the very beginning. We might say, however, that the mimoceran character of the open coil is pushed back by unequal acceleration and lost, while other mimoceran characters are retained, though so merged with those of *Anarcestes* that it is impossible to recognize them.

Each ammonite went through a larval history that is long and varied in direct proportion to the length of time from its period back to the Lower Devonian, when the first of the race are known. Thus in the *Nautilinidae*, the first group of ammonoids, the ontogeny is comparatively simple, there being few

<sup>1</sup> Balfour, Treatise on Comparative Embryology, vol. ii, p. 362.

<sup>2</sup> "Die Fauna mit *Maeneceras terebratum* Sandberger," *Abhandl. k. Preussischen Geol. Landesanstalt*, N.F., Heft 16, p. 77, Pl. III, Figs. 15-20, 1895.

<sup>3</sup> "Notes on the Early Stages of Certain Goniatites," *16th Ann. Rep. State Geologist of New York*, pp. 165-168, 1898.

changes from the larval period up to maturity. But the higher Devonian and Carboniferous forms go through several generic changes before they reach maturity, while Mesozoic genera have still longer larval and adolescent periods,—that is, longer in the sense of going through more stages. In Paleozoic species, however, one rarely finds ammonoids preserved so that the inner coils may be separated. In Mesozoic species, while the preservation is often good, the acceleration is usually so great that any certain interpretation of the meaning of larval stages is difficult, not to say impossible.

*Method of phylogenic research.*—Since ammonites preserve in each individual a complete record of their ontogeny, one might work out the life history of each species from a single specimen by making drawings of each stage before pulling off the coils representing this stage. In some few cases the writer has succeeded in taking off the outer coils so as to show almost the complete ontogeny in a single specimen without destroying the specimen. But this method usually necessitates the destruction of those parts that are taken off, and so the original is lost, and the naturalist has to show for his work only his notes and drawings, which may or may not be sufficiently accurate; his results cannot be verified.

The more satisfactory way is to select a number of well-preserved adults, so as to be sure of the identification of the species and to break off the outer coils until the desired stages are reached. To do this, finger nails and steel dental chisels are all the tools needed. After the specimen is reduced to a small size the coils are pulled off under water to prevent loss. The material used must be selected with great care, preferably limestone, not so soft as to crumble nor so hard as to shatter.<sup>1</sup> The young ammonite may be studied under the microscope in three different mountings: dry on white cardboard to see the surface markings; on white cardboard in a drop of water to see the septa and shape; under water in a watch-glass over a strong

<sup>1</sup> The results given in this paper are based on the study of about 150 specimens, illustrating all the life history of *Schloenbachia oregonensis*, but of course these could not all be figured, nor even included in the tables. Only the distinct stages and not the transitions were selected for illustration.

condensing lens to see the siphon and other internal characters when the specimen is translucent. This latter mounting is well suited to work in direct sunlight with a polarizing microscope, for the whole field is dark except where the doubly refracting calcite of the young ammonite allows the light to pass through.

#### NOMENCLATURE OF STAGES OF GROWTH.

In order to correlate ontogenic stages with generic changes seen in the development of the race it is necessary to have an exact scientific nomenclature. The most satisfactory, and one now being generally adopted, is that given by Professor Hyatt in "Phylogeny of an Acquired Characteristic."<sup>1</sup>

TABLE OF ONTOGENIC STAGES.

Stages.	Stages.	Substages.	Comparison with Phylogeny.
Embryonic (1)	Embryonic	{ Protembryo Mesembryo Metembryo Neoembryo Typembryo Phylembryo	Phylembryonic
Larval (2)	Nepionic	{ Ananepionic Metanepionic Paranepionic	Phylonepionic
Adolescent (3)	Neanic	{ Ananeanic Metaneanic Paraneanic	Phyloneanic
Adult (4)	Ephebic	{ Anephebic Metephebic Parephebic	Phylephebic
Senile (5)	Gerontic	{ Anagerontic Metagerontic Paragerontic	Phylogerontic
			Epacme
			Acme
			Paracme

With the embryonic stage the paleontologist can do nothing, except the very last substage, or phylembryo, when the *Mollusca*, *Brachiopoda*, and other groups begin to secrete their shells; but all the later stages are easily accessible in well-preserved material.

<sup>1</sup> *Proc. Amer. Phil. Soc.*, vol. xxxii, No. 143, pp. 391 and 397.



The best example of correlation of ontogenetic stages with phylogeny is the genealogy of *Medlicottia*, worked out by Karpinsky, who has shown that the Carboniferous genus *Pronorites* goes through the following stages: latisellate protoconch, phyl-embryonic; with the second suture it reaches the *Anarcestes* stage, nepionic; about the end of the first revolution the *Ibergiceras* stage begins, paranepionic; second revolution shows the *Paraprolecanites* stage, neanic; on the third whorl begins the *Pronorites* stage, adult. Thus with regard to *Pronorites* the genus *Anarcestes* is phylonepionic, *Ibergiceras* is phyloparanepionic, *Paraprolecanites* is phyloneanic. In the same work Karpinsky has shown that *Medlicottia* is a direct descendant of *Pronorites* and in its development goes through all the stages of the ancestral genus and adds several more. The first revolution of *Medlicottia* could not be studied, but on the second revolution was seen the *Ibergiceras* stage, metanepionic; on the third whorl the *Paraprolecanites* stage, paranepionic; at end of the third whorl the *Pronorites* stage, beginning of the neanic; on the fourth whorl the *Sicanites* stage, end of the neanic; on the fifth whorl the *Promedlicottia* stage, anephebic; and lastly, at end of the fifth whorl, *Medlicottia*, adult in characteristics, though not yet in size.

Genus SCHLOENBACHIA Neumayr, *Sitzungsberichte k. Akad. Wiss. Wien* (Math. Nat. Kl.), Bd. lxxi, 1. Abth., p. 658, 1875.

As originally defined by Neumayr, *Schloenbachia* was to include forms with narrow, compressed whorl, strong curved lateral ribs, a sharp, often notched, keel; septa comparatively little branched, two lateral, and one distinct auxiliary lobe. The genus was supposed to be descended from *Amaltheus*, although Neumayr<sup>1</sup> says that we can only assign *Schloenbachia* with probability to this group, since it appears suddenly in the Cretaceous as an immigrant, without local ancestors; it has later been broken up into a number of genera and subgenera of questionable value, some of which cannot be sharply differentiated from each other.<sup>2</sup>

<sup>1</sup> *Loc. cit.*, pp. 654 and 658.

<sup>2</sup> F. B. Meek, "Report on Invert. Cretac. Foss. Upper Missouri," 1876. Gros-souvre, "Les Ammonites de la craie supér. de la France," 1893.

K. A. von Zittel<sup>1</sup> has separated *Schloenbachia* from the *Amaltheidae*, and placed it in a family of its own, the *Prionotropidae*; which change is quite proper, for *Schloenbachia* does not go through in its adolescent period any stages corresponding either to *Amaltheus* or *Oxynoticeras*. Zittel regards the *Prionotropidae* as an offshoot of the *Amaltheidae*, and these in turn from the *Prolecanitidae*; but neither *Schloenbachia* nor the *Amaltheidae* go through larval stages corresponding to this Paleozoic group, but rather to the *Glyphioceratidae*.

SCHLOENBACHIA OREGONENSIS Anderson ms., Pls. A-E.

*Schloenbachia* sp. indet., aff. *S. chicoensis* Trask; J. P. Smith, *Journ. Geol.*, Pl. A, Figs. 1-7, vol. v, No. 5, p. 521, 1897.

*Schloenbachia* sp. indet., J. P. Smith, Chapter IX in Jordan's "Footnotes to Evolution," Pl. C, Figs. 1-11.

The adult is narrow, discoidal, high-whorled, with wide, shallow umbilicus, and almost parallel sides. The whorls embrace about two-fifths of the preceding. The surface is ornamented with strong ribs that branch in groups of two from strong knots on the umbilical shoulders, bend forward and form smaller knots on the angular abdominal shoulders, and then turn forward in a sharp angle to the keel. These ribs are exceedingly variable, sometimes fine, and sometimes coarse, with transitions from one to the other. Between these bundles of ribs there are from one to two single ribs that do not reach the umbilical shoulders. The keel is rather low, sharp, and slightly notched by the ribs; the sloping space between the keel and the abdominal shoulders has no furrow, although the row of abdominal knots may give that impression. A cross-section of the adult is shown on Pl. C, Fig. 7, diameter 22.25 mm., six whorls, on which the increasing relative height and flattening sides of the whorls may be seen. The septa are comparatively simple, and not very digitate; they show a wide external lobe divided by a short and broad siphonal saddle; a deep, broad, first lateral lobe; second lateral lobe about one-half as deep as the first; and a shallow auxiliary lobe. The first lateral saddle is notched rather deeply near the middle, a character that begins

<sup>1</sup> *Grundzüge d. Palaeontologie*, p. 430, 1895.

in the early youth of the shell and continues to increase until the adult stage is reached. The septa of a specimen at diameter 18.50 mm. are shown on Pl. E, Fig. 5.

*S. oregonensis* grows to a diameter of at least 30 mm., although no perfect specimens of that size were obtained. The measurements of an adult at end of the sixth whorl are as follows :

	MM.
Diameter . . . . .	22.25 = 1.00
Height of the last whorl . . . . .	9.00 = 0.40
Height of last whorl from the top of the preceding	7.28 = 0.32
Width of last whorl . . . . .	5.00 = 0.22
Involution . . . . .	1.72 = 0.07
Width of umbilicus . . . . .	7.64 = 0.34

This species is nearest to *Schloenbachia chicoensis* Trask, *Proc. Calif. Acad. Sci.*, vol. i, p. 92, Pl. II, Fig. 1, 1856; and *Palaeontol. Calif.*, vol. i, p. 68, Pl. XIII, Fig. 17, and Pl. XIV, Fig. 17, to which it was doubtfully referred by Mr. F. M. Anderson.<sup>1</sup>

*S. chicoensis*, as figured by Gabb, has narrower and more involute whorls than *S. oregonensis*, flatter sides, and stronger nodes on the shoulder keels, and also has the shoulder keels nearly as high as that on the abdomen. Through the kindness of Dr. J. C. Merriam, of the University of California, the writer was able to examine a series of *Schloenbachia chicoensis*, as figured and described by Gabb; the following are the dimensions of a typical specimen :

	MM.
Diameter . . . . .	24.0
Height of last whorl . . . . .	12.0
Width of umbilicus . . . . .	5.0

The dimensions of a specimen nearing the end of the adolescent stage are as follows :

*S. chicoensis* (as identified by Gabb),

	MM.
Diameter . . . . .	13.00 = 1.00
Height of the last whorl . . . . .	5.3 = 0.40
Height of last whorl from the preceding . . . . .	4.3 = 0.33
Width of last whorl . . . . .	3.5 = 0.26
Involution . . . . .	1.0 = 0.07
Width of umbilicus . . . . .	4.0 = 0.30

<sup>1</sup> *Journ. Geol.*, vol. iii, No. 4, p. 467.

The adolescent *S. chicoensis* resembles in appearance and in relative measurements the adults of *S. oregonensis*, and very probably is a descendant of the latter species.

*Occurrence and locality.*—The material on which this paper is based was collected by Mr. Frank M. Anderson, at the Forty-Nine mine, one and one-half miles southwest of Phoenix, Oregon, in beds supposed to belong to the Upper Horsetown formation, top of the Lower Cretaceous, and described by him in "Some Cretaceous Beds of the Rogue River Valley, Oregon."<sup>1</sup> The writer's thanks are especially due Mr. Anderson for the generosity with which he furnished the specimens to illustrate this work. In a forthcoming paper, in the *Proceedings of the California Academy of Science*, Series 3, Mr. Anderson will figure and describe *Schloenbachia oregonensis* and the rest of this interesting fauna.

#### ONTOGENIC STAGES.

##### *Nepionic or Larval.*

*Phylembryonic.*—The early embryonic stages are shell-less, and necessarily cannot be represented in fossils, so the paleontologist begins his investigations with the phylembryonic, when the shell gland becomes functional, and the class or phylum can be made out. This is represented in the ammonites by the protoconch, which in this species is a smooth, oval, bobbin-shaped body, a little wider than high, to which the chambered coil is attached. With this stage begins the siphon, as a pear-shaped sac, or *caecum*, taking up a large part of the entrance from the protoconch to the chambered shell. The dimensions of the protoconch are remarkably constant in a large number of specimens; those of the protoconch figured on Pl. A, Figs. 1-3, are as follows:

	MM.		MM.
Diameter . . .	0.42	Width . . .	0.48

This stage is analogous to the *protegulum* of the brachiopods, *protaspis* of the trilobites, and *prodissoconch* of the pelecypods, and corresponds to the primitive cephalopod. The embryonic

<sup>1</sup> *Journ. of Geol.*, vol. iii, No. 4.



shell probably included part of the spiral chamber, but for want of a natural indication of the end of the stage, the phylembryonic is arbitrarily limited to the protoconch.

*Ananepionic.* — With the formation of the first septum the animal is considered to cease to be an embryo and to begin its larval history. This, of course, is purely arbitrary, since the ammonites are all extinct and we have no way of knowing at what stage they left the egg. At this period the siphon, which is in the center, takes up nearly half of the height of the whorl. The first septum consists of a broad, long, abdominal saddle, a pair of rather narrow lateral lobes, and a pair of short, narrow saddles on the umbilical shoulders. This is shown on Pl. A, Fig. 3, and Pl. C, Fig. 1. It is distinctly nautilian and corresponds to some Silurian nautiloid genus, although it is not possible to say which one, because the characters are not distinctive enough. The internal part of the septum is nearly straight, showing no lobes nor saddles.

*Metanepionic.* — The second larval substage begins at the second septum, when the whorl is low, broad, and deeply embracing. Pl. C, Fig. 1, shows that at the second septum the broad, abdominal saddle is divided by a deep and broad ventral lobe; at this stage the shell resembles the Lower Devonian *Anarcestes*, one of the first of the typical ammonoids. At the third and fourth septa little change takes place, but these probably correspond to *Parodoceras* and *Prionoceras* of the Devonian. At the fifth septum the ventral lobe broadens, showing a transition from *Prionoceras* to *Glyphioceras* (or *Goniatites* s. str.). The ananepionic and metanepionic substages take up the first quarter of a coil. The siphon, during this substage, is still median, and remains so up to three-quarters of a whorl, when the paranepionic stage is well along, but always decreasing in relative diameter as compared with the height of the successive chambers. The form of the shell at the metanepionic stage is shown in the first quarter of a coil from the protoconch, on Pl. A, Figs. 4 and 5; the septa are shown on Pl. C, Fig. 1, at the second, third, fourth, and fifth. On some specimens the metanepionic substage ended with the fifth septum.

*Paranepionic*. — Hyatt<sup>1</sup> says that the paranepionic substage in the later ammonoids begins with the division of the ventral lobe, and continues as long as only goniatite characters are shown. In *Schloenbachia oregonensis* the sixth septum has a divided ventral lobe and two lateral lobes, like *Glyphioceras* (or *Goniatites* s. str.). This is shown on Pl. C, Fig. 1. If we follow Hyatt's definition the paranepionic stage will have to be subdivided, for there are three well-marked goniatite stages in it, the *Glyphioceras*, *Gastrioceras*, and *Paralegoceras* stages. In a recent paper<sup>2</sup> the writer has shown that *Glyphioceras* in its ontogeny goes through as a larva the stages *Anarcestes* and *Tornoceras* (*Parodoceras*); as a youth it is a *Prionoceras*, and takes on its own characters at a diameter of about 6 mm. J. M. Clarke<sup>3</sup> says that *Tornoceras* and *Parodoceras* are distinct genera, but that they appear along with *Anarcestes* early in the Devonian, and, therefore, are probably not descendants from that genus, but have a common origin with it. If this is the case the genealogy of the *Glyphioceratidae* will have to be revised, as will also the nomenclature of the chief genus of the family, for E. Haug<sup>4</sup> has recently shown that *Goniatites* de Haan must be retained for the group of *G. sphaericus* Martin, while *Glyphioceras* Hyatt may be retained for the group of *G. diadema*.

*Glyphioceras* stage. — Now *Schloenbachia oregonensis* goes through these same preliminary stages, but is so greatly accelerated that it reaches the glyphioceran stage at the end of the first quarter of a coil from the protoconch, and at the sixth septum, as shown on Pl. C, Fig. 1. The early part of this stage is shown on Pl. A, Figs. 4 and 5, one-half coil, first eight septa, and diameter 0.58 mm.; Fig. 6 shows a little more advanced glyphioceran stage, development of the septa from the third to the tenth, diameter 0.64 mm.; Figs. 7 and 8 show

<sup>1</sup> "Phylogeny of an Acquired Characteristic," p. 416.

<sup>2</sup> *Proc. Calif. Acad. Sci.*, Series 3, vol. i; *Geol.*, No. 3, 1897, "Development of *Glyphioceras*," etc.

<sup>3</sup> "Naples Fauna (Fauna with *G. Intumescens*) in Western New York," *16th Ann. Rep. State Geologist of New York*, p. 109, 1898.

<sup>4</sup> "Études sur les Goniatites," *Mém. 18, Paléontologie, Soc. Géol., France*, 1898, p. 27.



it with nine septa, diameter 0.68 mm., and three-quarters of a coil; Fig 9 shows this same stage at a little over three-quarters of a coil, diameter 0.74 mm., and its septa are shown on Pl. D, Fig. 1. These figures show a gradually increasing height of the whorl as compared with the width. The glyphioceran stage lasts up to a diameter of 1 mm., and about one and one-quarter coils, near the end of which stage, at diameter of 0.80 mm., a deep sulcation or constriction makes its appearance; this distinctively glyphioceran character was observed on a large number of specimens near the end of the first whorl, and never after that.

*Gastrioceras* stage. — Near the beginning of the second whorl, at diameter of a little over 1 mm., and after the appearance of the constriction, the umbilicus begins to widen, until at diameter of 1.20 mm. it is proportionally wider than in any species of *Glyphioceras*; this is shown on Pl. A, Figs. 10 and 11, one and three-eighths coils, and is transitional to *Gastrioceras*, a genus especially characteristic of the Upper Carboniferous. A somewhat larger specimen, diameter 1.33 mm., one and five-eighths whorls, is shown on Pl. A, Figs. 12 and 13. As the size increases the shape becomes more decidedly gastrioceran, as shown on Pl. A, Figs. 14 and 15, one and seven-eighths coils, diameter 1.65 mm.; the septa of this are seen on Pl. D, Fig. 2. This stage corresponds to that group of *Gastrioceras* that lacks the umbilical ribs and has the second lateral lobe on the sides of the shell, as in *Gastrioceras illinoisense* Miller and Gurley,<sup>1</sup> of the Coal Measures.

*Paralegoceras* stage. — The gastrioceran stage lasts from near the beginning of the second whorl, diameter a little over 1 mm., up to two and one-eighth whorls, diameter 2.15 mm., when a third lateral lobe appears on the umbilical border; then the whorl becomes higher and narrower, and the whole aspect of the shell is like *Paralegoceras* Hyatt, a genus especially diagnostic of the Upper Carboniferous, and supposed to be a direct descendant of *Gastrioceras*.<sup>2</sup> This stage is shown

<sup>1</sup> Bulletin XI, Illinois State Mus., N. H., p. 42, Pl. V, Figs. 6-8, 1896.

<sup>2</sup> For the relations of *Glyphioceras*, *Gastrioceras*, and *Paralegoceras*, see paper by the writer, "Marine Fossils from the Coal Measures of Arkansas," *Proc. Amer. Phil. Soc.*, vol. xxxv, No. 152, 1896.

on Pl. B, Figs. 1 and 2, and the septa on Pl. D, Fig. 4, although the third lateral lobe is considerably exaggerated, on account of a mistake in drawing. But even if the third lateral lobe were entirely lacking the stage might still be referred to *Paralegoceras*, according to the usage of Hyatt. Throughout this, as in all preceding stages, each coil embraces about two-fifths of the preceding. By reference to the table of stages of growth, the widening umbilicus and flattening whorl may be traced just as in the drawings of the successive stages. This substage is short, lasting only half a coil, from diameter 2.15 mm. up to two and five-eighths whorls, diameter 2.70 mm.

The decrease in relative size of the siphon in the larval stages may be seen from the following figures :

At the first septum the siphon is 48 per cent of height of the whorl.					
At one-quarter of a coil	"	"	35	"	"
" one-half	"	"	32	"	"
" three-quarters	"	"	25	"	"
" one and one-quarter coils	"	"	24	"	"
" one and one-half	"	"	23	"	"
" one and three-quarters coils	"	"	22	"	"
" two coils	"	"	20	"	"
" two and one-half coils	"	"	17	"	"

#### NEANIC OR ADOLESCENT.

*Ananeanic*. — When an ammonite in its development has taken on characters that the goniatites never had, it may be said to have completed the larval stage and to have begun the adolescent. At the end of the *Paralegoceras* stage, diameter 2.70 mm., about the middle of the third whorl, the abdomen becomes sharpened and somewhat higher, and a keel appears. The smooth sides, simple goniatitic septa, and ventral keel all remind one of the Triassic genus *Styrites*<sup>1</sup> Mojsisovics. This stage is shown on Pl. B, Figs. 3 and 4, diameter 3.10 mm., three whorls, with the beginning of the keel at diameter 2.70 mm.; the septa are seen on Pl. D, Fig. 4.

<sup>1</sup> "Das Gebirge um Hallstadt," *Abhandl. k. k. Geol. Reichsanstalt*, Wien, Bd. vi, p. 264, 1893. The ontogeny of this genus is not described here, and we do not know that it really goes through the preliminary development of the *Glyphioceratidae*.

*Schloenbachia oregonensis* remains in this stage about a quarter of a revolution, up to the diameter 3.15 mm., two and seven-eighths whorls; then without any other change in characters the first lateral saddle suddenly becomes indented, as shown on Pl. B, Figs. 5 and 6, diameter 3.71 mm., and the projection of the septa on Pl. D, Fig. 5. This stage does not correspond to any known genus, but the characters have the nature of Lower Triassic genera, and so it may be referred to some unknown form of that age; it may be provisionally called the *Parastyrites* stage. At diameter of 4.00 mm. the rounded abdominal shoulders become angular, forming keels. The *Parastyrites* stage lasts about half a revolution, to near the middle of the fourth whorl.

*Metaneanic*.—At diameter 4.5 mm., three and three-eighths whorls, ribs appear suddenly on the sides, faint at first, but rapidly becoming distinct; this is figured on Pl. B, Fig. 7, diameter 5.60 mm., three and seven-eighths whorls. At first the ribs, which branch out from nodes on the umbilical shoulders, reach only to the abdominal angles. This stage usually ends with the fourth whorl, at diameter a little over 7 mm., thus lasting about five-eighths of a coil. Near the end of the fourth whorl the septa, which up to this time have persisted in their simple goniatic character, become slightly digitate, or ammonitic; this is shown on Pl. E, Fig. 1, diameter 6.00 mm., and Fig. 2, diameter 6.40, a little over four coils.

*Paraneanic*.—Near the beginning of the fifth whorl, at diameter between 7 and 8 mm., the ribs begin to form knots on the abdominal keels and the nodes on the umbilical shoulders grow stronger. The height of the whorl, in proportion to its width, grows more pronounced, and, instead of a sharpened abdomen with a keel, the abdominal shoulders become higher and more angular, and the ventral keel rises little above them. At the same time the septa become more ammonitic, as shown on Pl. E, Fig. 3, diameter 8 mm., four and one-half whorls, and Fig. 4, diameter 9.20 mm., four and three-quarters whorls. This stage lasts up to a diameter of about 12 mm., five whorls. The ribs and the nodes on the abdominal shoulder keels become gradually stronger, and the whorl grows

TABLE OF STAGES OF GROWTH.

	PHYLEM-BRYONIC.	NEPHONIC OR LARVAL.									
		ANA-, TO META-, TO PARANEPHONIC.					PARANEPHONIC.				
		<i>Glyptoceran</i> substage.	<i>Glyptoceran</i> substage.	<i>Glyptoceran</i> substage.	<i>Glyptoceran</i> substage.	<i>Glyptoceran</i> substage.	<i>Glyptoceran</i> substage.	<i>Glyptoceran</i> substage.	<i>Glyptoceran</i> substage.	Transition from <i>Glyptoceran</i> to <i>Gastricoceran</i> .	
	<i>Protoconch</i> .	1/4 whorl.	1/8 whorl.	1/4 whorl.	1/2 whorl.	3/4 whorl.	1 whorl.	1 1/4 whorls.	1 1/2 whorls.	1 3/4 whorls.	
Diameter .....	mm.	0.42 = 1.00	0.64 = 1.00	0.68 = 1.00	0.73 = 1.00	0.73 = 1.00	0.90 = 1.00	1.08 = 1.00	1.17 = 1.00	mm.	mm.
Height of last whorl .....		0.58 = 0.34	0.25 = 0.40	0.28 = 0.41	0.29 = 0.39	0.29 = 0.39	0.37 = 0.40	0.41 = 0.38	0.43 = 0.37	0.40 = 0.33	1.20 = 1.00
Height of last whorl from the preceding .....		0.34 = 0.80	0.23 = 0.40	0.25 = 0.39	0.25 = 0.39	0.25 = 0.39	0.37 = 0.40	0.41 = 0.38	0.43 = 0.37	0.40 = 0.33	0.40 = 0.33
Width of last whorl .....		0.08 = 0.19	0.16 = 0.27	0.15 = 0.23	0.20 = 0.29	0.20 = 0.29	0.26 = 0.29	0.31 = 0.29	0.33 = 0.25	0.33 = 0.27	0.33 = 0.27
Involution .....		0.48 = 1.14	0.55 = 0.94	0.58 = 0.90	0.54 = 0.79	0.53 = 0.72	0.56 = 0.61	0.62 = 0.59	0.59 = 0.51	0.63 = 0.52	0.63 = 0.52
Width of umbilicus .....	—	0.07 = 0.12	0.10 = 0.16	0.10 = 0.14	0.10 = 0.12	0.09 = 0.13	0.09 = 0.10	0.10 = 0.09	0.13 = 0.12	0.07 = 0.06	0.07 = 0.06
		0.17 = 0.29	0.13 = 0.20	0.15 = 0.22	0.14 = 0.19	0.14 = 0.19	0.25 = 0.27	0.34 = 0.31	0.43 = 0.37	0.49 = 0.40	0.49 = 0.40

		NEPHONIC OR LARVAL.									
		PARANEPHONIC.									
		<i>Gastricoceran</i> substage.	<i>Gastricoceran</i> substage.	<i>Gastricoceran</i> substage.	<i>Gastricoceran</i> substage.	<i>Gastricoceran</i> substage.	<i>Gastricoceran</i> substage.	End of <i>Gastricoceran</i> substage.	<i>Paratrocera</i> substage.		
		1 1/8 whorls.	1 1/4 whorls.	1 1/2 whorls.	2 whorls.	2 1/4 whorls.	2 1/2 whorls.	2 3/4 whorls.	2 3/4 whorls.	2 3/4 whorls.	2 3/4 whorls.
Diameter .....	mm.	1.33 = 1.00	1.52 = 1.00	1.65 = 1.00	1.72 = 1.00	1.93 = 1.00	2.08 = 1.00	2.26 = 1.00	2.43 = 1.00	2.71 = 1.00	mm.
Height of last whorl .....		0.48 = 0.34	0.55 = 0.36	0.61 = 0.36	0.62 = 0.36	0.63 = 0.37	0.71 = 0.33	0.81 = 0.35	0.89 = 0.36	0.95 = 0.35	mm.
Height of last whorl from the preceding .....		0.36 = 0.26	0.46 = 0.30	0.51 = 0.30	0.52 = 0.30	0.56 = 0.29	0.57 = 0.27	0.66 = 0.29	0.70 = 0.28	0.74 = 0.27	mm.
Width of last whorl .....		0.66 = 0.49	0.65 = 0.42	0.70 = 0.46	0.81 = 0.47	0.85 = 0.44	0.89 = 0.43	0.86 = 0.38	0.92 = 0.38	0.95 = 0.35	mm.
Involution .....		0.12 = 0.09	0.12 = 0.07	0.10 = 0.06	0.10 = 0.06	0.12 = 0.08	0.14 = 0.06	0.15 = 0.07	0.19 = 0.08	0.21 = 0.08	mm.
Width of umbilicus .....		0.50 = 0.37	0.62 = 0.41	0.59 = 0.35	0.66 = 0.38	0.78 = 0.40	0.87 = 0.41	0.90 = 0.40	0.93 = 0.38	1.11 = 0.40	mm.

	NEANIC OR ADOLESCENT.						EPHEBIC OR ADULT.		
	ANANEANIC.			METANEANIC.		PARANEANIC.	ANEPIHEBIC.	METEPHEBIC.	
	<i>Styrites</i> stage.	<i>Parastyrites</i> stage.	<i>Parastyrites</i> stage.	3 $\frac{1}{2}$ whorls.	4 $\frac{1}{2}$ whorls.	4 $\frac{1}{2}$ whorls.	5 whorls.	5 $\frac{1}{2}$ whorls.	6 whorls.
	2 $\frac{1}{2}$ whorls.	2 $\frac{1}{2}$ whorls.	3 $\frac{1}{2}$ whorls.	mm.	mm.	mm.	mm.	mm.	mm.
Diameter.....	2.90 = 1.00	3.18 = 1.00	3.71 = 1.00	5.60 = 1.00	7.10 = 1.00	9.20 = 1.00	12.40 = 1.00	15.40 = 1.00	22.25 = 1.00
Height of last whorl.....	1.02 = 0.35	1.14 = 0.35	1.26 = 0.34	1.76 = 0.37	2.60 = 0.36	3.50 = 0.35	4.40 = 0.37	5.40 = 0.38	7.90 = 0.40
Height of last whorl from the preceding.....	0.79 = 0.27	0.99 = 0.31	1.09 = 0.27	1.73 = 0.38	2.60 = 0.26	3.50 = 0.30	4.40 = 0.30	5.40 = 0.28	7.28 = 0.32
Width of last whorl.....	1.07 = 0.36	1.04 = 0.32	1.22 = 0.32	1.73 = 0.38	1.99 = 0.28	2.37 = 0.25	3.00 = 0.23	3.85 = 0.25	5.90 = 0.22
Involution.....	0.23 = 0.08	0.15 = 0.05	0.25 = 0.08	0.37 = 0.07	0.79 = 0.09	0.70 = 0.08	0.90 = 0.07	1.50 = 0.10	1.72 = 0.07
Width of Umbilicus.....	1.14 = 0.39	1.22 = 0.38	1.41 = 0.38	2.14 = 0.38	2.82 = 0.39	3.80 = 0.41	5.00 = 0.40	5.40 = 0.35	7.64 = 0.34



CROSS-SECTION. ADOLESCENT STAGE. (Figured on Plate C, Fig. 6.)

	EMBRY- ONIC.	NEPHONIC OR LARVAL.				NEANIC OR ADOLESCENT.			
		Glyphioceras.		Gastrioceras.		Parale- goeras.		Parastyriles.	
		Phylem- bryo.							
		Proto- conch.							
Diameter .....	mm.	$\frac{1}{2}$ coil.	1 coil.	$1\frac{1}{2}$ coils.	2 coils.	$2\frac{1}{2}$ coils.	3 coils.	$3\frac{1}{2}$ coils.	4 coils.
Height of last whorl .....	0.46	0.56	0.88	mm.	mm.	mm.	mm.	mm.	mm.
Height of last whorl from the preceding .....	0.20	0.25	0.35	1.21	1.64	2.36	3.30	4.52	6.25
Width of last whorl .....	0.09	0.17	0.26	0.41	0.55	0.83	1.13	1.53	2.24
Width of last whorl from the preceding .....	0.48	0.50	0.54	0.63	0.75	0.86	0.93	1.23	1.81
Involution .....	0.11	0.08	0.09	0.08	0.10	0.16	0.20	0.30	0.43
Width of umbilicus .....	—	0.13	0.28	0.45	0.68	1.00	1.33	1.86	2.50

CROSS-SECTION. ADULT. (Figured on Plate C, Fig. 7.)

		NEPHONIC OR LARVAL.				NEANIC OR ADOLESCENT.				EPHEBIC OR ADULT.	
		Glyphioceras.		Gastrioceras.		Parale- goeras.		Styr- yles.		Para- styriles.	
Diameter .....	mm.	$\frac{1}{2}$ coil.	1 coil.	$1\frac{1}{2}$ coils.	2 coils.	$2\frac{1}{2}$ coils.	3 coils.	$3\frac{1}{2}$ coils.	4 coils.	$4\frac{1}{2}$ coils.	5 coils.
Height of last whorl .....	0.65	0.90	1.23	1.61	2.25	mm.	mm.	mm.	mm.	mm.	mm.
Height of last whorl from the preceding .....	—	—	—	—	—	—	—	—	—	—	—
Width of last whorl .....	—	—	—	—	—	—	—	—	—	—	—
Width of last whorl from the preceding .....	—	—	—	—	—	—	—	—	—	—	—
Involution .....	—	—	—	—	—	—	—	—	—	—	—
Width of umbilicus .....	—	—	—	—	—	—	—	—	—	—	—

Schönbachia.

mm.

15.40

22.25

9.00

5.90

7.28

5.00

3.85

1.72

1.50

1.72

7.64











steadily higher and narrower, changing gradually to the adult characters, but with no sudden change to mark the stage.

#### EPHEBIC OR ADULT.

It would be purely artificial to divide the adult stage into the three subdivisions ana-, meta-, and paraphebic, for the change is too gradual. Near the beginning of the sixth whorl, at a diameter of 12 mm., the nodes on the shoulder keels grow stronger and form continuations of the ribs, bending forwards over the intervening space to the ventral keel, and finally notching it. Since most species of *Schloenbachia* have this character this may be considered as the beginning of the adult period. These characters are seen sometimes as early as four and three-quarters whorls, diameter of 10 to 11 mm. A general description of the adult stage has already been given under the diagnosis of the species. The adult septa are figured on Pl. E, Fig. 5, at diameter 18.20 mm., and a cross-section of an adult specimen on Pl. C, Fig. 7, diameter 22.25 mm.

#### SYNOPSIS OF RESULTS.

*Schloenbachia oregonensis* is a remarkable species, in showing its descent so well through its ontogeny; the only other species of which larval stages have been figured, *S. varicosa* Sowerby, figured by Branco in *Palaeontographica*, vol. xxvi, Pl. E, Fig. 4, shows the glyphioceran character at the third septum, the *Anarcestes*, *Tornoceras*, and *Prionoceras* stages being omitted by acceleration of development. The omission of stages occurs just at this point, between the protoconch, which is always constant in any one group, and the larval stages. A kindred form, *Oxynticeras oxynotum*, reaches the glyphioceran stage at the second septum, having skipped the preceding stages, but going through the *Gastrioceras*, *Paralegoceras*, and *Styrites* stages just as does *Schloenbachia oregonensis*. This seems to the writer to have been due to the hatching of different genera or species at different stages of growth, the omitted stages corresponding to a period when the animal remained in the egg after formation of the protoconch.

*Schloenbachia oregonensis* in its development repeats the history of *Anarcestes*, *Parodoceras*, and *Prionoceras* in the first five septa and one-quarter of a coil from the nautiloid protoconch; then for about one whorl it is a *Glyphioceras*; for about one and one-quarter whorls it is a *Gastrioceras*; then for a little more than one-quarter of a revolution it is a *Paralogoceras*, and at two and five-eighths coils ends its goniatite history, takes on a keel, and becomes an ammonite, but one like the simpler ammonites of the Permian and Lower Trias. The ananeanic stage lasts up to three and three-eighths whorls, that is, about three-quarters of a revolution; the metaneanic stage lasts up to the end of the fourth whorl, and the paraneanic to near the end of the fifth whorl. With the beginning of the sixth whorl, at diameter of about 12 mm., the shell begins to take on its own proper characters, and is then in the ephebic stage, although adults grow to at least 30 mm. in diameter, and probably larger.

The larval stages may be compared with considerable certainty to ancestral Paleozoic genera, but the Mesozoic genera to which the adolescent stages might be compared are probably mostly unknown as yet, although they will be found among trachyostracan descendants of the *Glyphioceratidae*, and not among the *Prolecanitidae*.

No more striking demonstration of the law of acceleration of development, or tachygenesis, is possible than where a shell in its larval history hastens through, in two and five-eighths whorls, and in growth up to 2.70 mm., generic changes from *Anarcestes*, *Parodoceras*, *Prionoceras*, *Glyphioceras*, *Gastrioceras*, and *Paralogoceras*, an amount of development that its ancestors required the time from the Lower Devonian to the end of the Carboniferous to accomplish. In the succeeding adolescent stages the changes are not nearly so rapid.

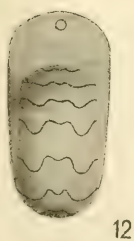
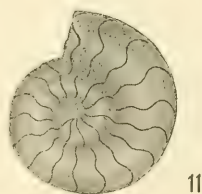
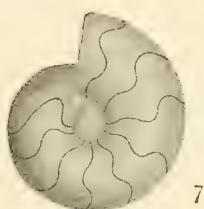
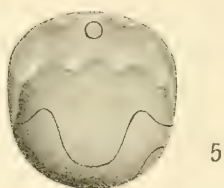
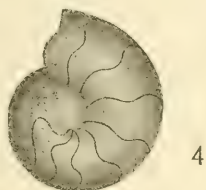
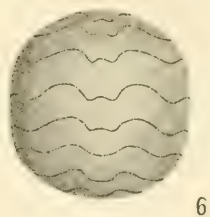
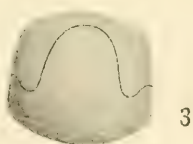
Another fact brought out by the investigation of many specimens is that individual variation increases greatly with the advance of the stage. Thus all protoconchs and most chambered stages are alike up to the end of the larval period. After that the uniformity ends, for in the adolescent period the ribs begin at various sizes, as does also the digitation of the septa. And in the acquirement of adult characters still greater



variation was observed, not only in time, but also in the characters themselves, so that one would be inclined to make several species out of one, were it not for the transitions between the varieties. A parallel study of the ontogeny of two nearly related species has shown just these same facts, only in greater degree, for specific variation is only individual variation carried to extremes.

## EXPLANATION OF PLATE A.

*Schloenbachia oregonensis* Anderson.FIGS. 1-3. Protoconch, phylembryonic to ananepionic.  $\frac{4}{1}^0$ .FIGS. 4 and 5. Phylembryonic to paranepionic; diameter 0.58 mm.; one-half whorl, first eight septa, glyphioceran stage at the sixth.  $\frac{4}{1}^0$ .FIG. 6. Paranepionic, glyphioceran substage; diameter 0.64 mm.; third to tenth septa, five-eighths of a whorl.  $\frac{4}{1}^0$ .FIGS. 7 and 8. Phylembryonic to paranepionic, glyphioceran substage; diameter 0.68 mm.; three-quarters of a whorl, nine septa.  $\frac{4}{1}^0$ .FIG. 9. Paranepionic, glyphioceran substage; diameter 0.74 mm.; seven-eighths of a whorl.  $\frac{4}{1}^0$ .FIGS. 10 and 11. Paranepionic, transition from glyphioceran to gastrioceran substages; diameter 1.20 mm.; one and three-eighths whorls.  $\frac{2}{1}^0$ .FIGS. 12 and 13. Paranepionic, transition from glyphioceran to gastrioceran substage; diameter 1.33 mm.; one and five-eighths whorls.  $\frac{2}{1}^0$ .FIGS. 14 and 15. Paranepionic, gastrioceran substage; diameter 1.65 mm.; one and seven-eighths whorls.  $\frac{2}{1}^0$ .

















## EXPLANATION OF PLATE B.

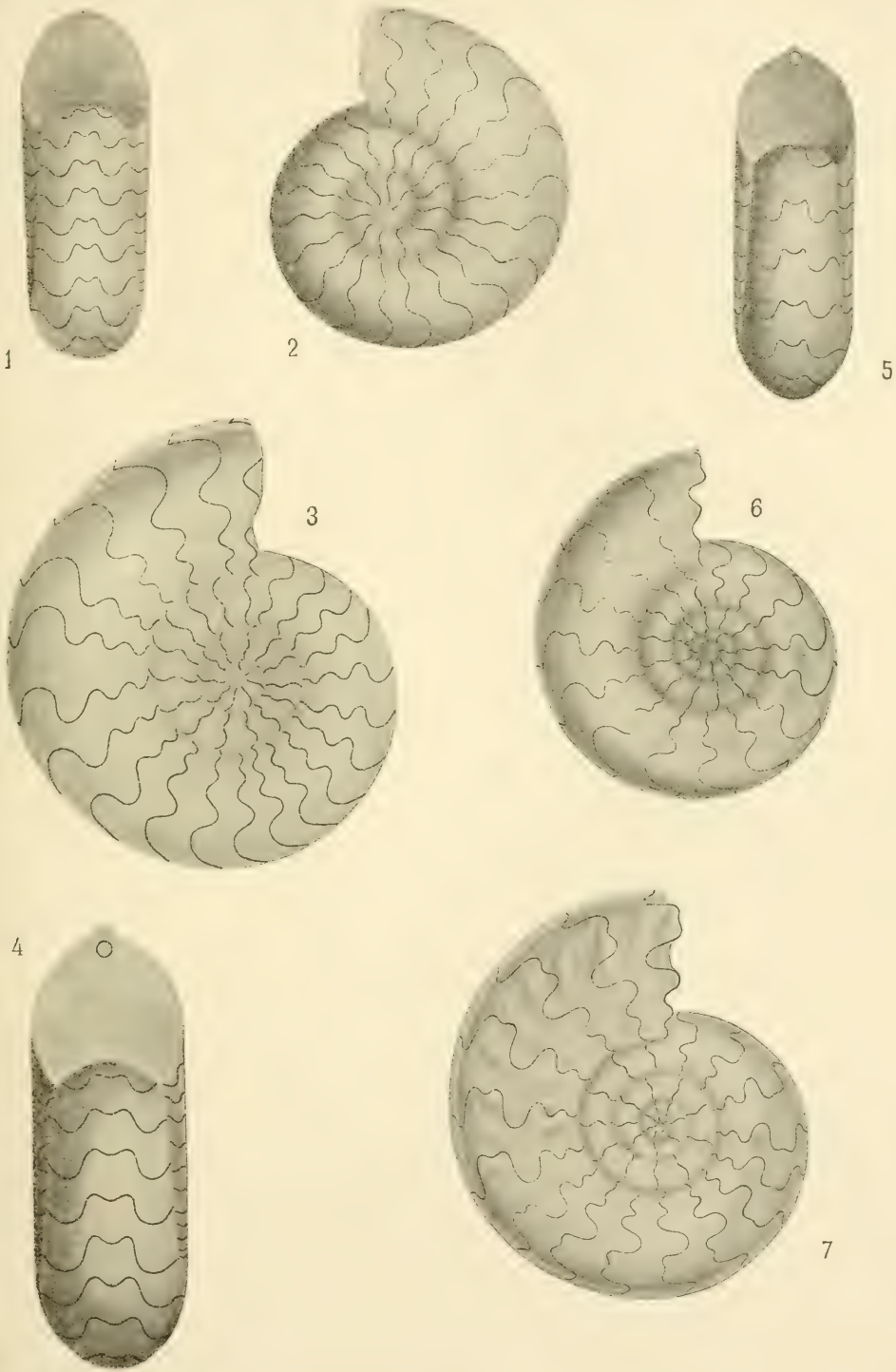
*Schloenbachia oregonensis* Anderson.

FIGS. 1 and 2. Paranepionic, paralegoceran substage; diameter 2.25 mm.; two and three-eighths whorls.  $\frac{2^0}{1}$ .

FIGS. 3 and 4. Ananeanic, *Styrites* stage; diameter 3.10 mm.; two and seven-eighths whorls.  $\frac{2^0}{1}$ .

FIGS. 5 and 6. Ananeanic, *Parastyrites* stage; diameter 3.70 mm.; three and one-fourth whorls.  $\frac{1^3}{1}$ .

FIG. 7. Metaneanic, advanced adolescent stage; diameter 5.60 mm.; three and three-quarters whorls, showing beginning of ribs at diameter 4.70 mm.  $\frac{1^0}{1}$ .









## EXPLANATION OF PLATE C.

*Schloenbachia oregonensis* Anderson.

FIG. 1. Protoconch of *Schloenbachia*, showing the first six sutures of the attached coil. Enlarged thirty times.

FIG. 2. Larval stage of *Schloenbachia*, diameter 0.68 mm.; thirty times enlarged; three-fourths of first whorl. 2a, side view; 2b, front view.

FIG. 3. Larval stage of *Schloenbachia*, diameter 0.64 mm.; thirty times enlarged. Showing sutures from the third to the tenth. From above.

FIG. 4. Larval stage of *Schloenbachia*, diameter 1.20 mm.; fifteen times enlarged; one and one-half whorls. 4a, front view; 4b, side view.

FIG. 5. End of larval stage of *Schloenbachia*, diameter 2.25 mm.; fifteen times enlarged. *Paralegoceras* stage. 5a, side view; 5b, front view.

FIG. 6. Cross-section of *Schloenbachia*, diameter 6.25 mm.; fifteen times enlarged; four whorls. Adolescent stage. The protoconch is seen in the center P.

FIG. 7. Cross-section of *Schloenbachia*, 22.25 mm.; three and one-half times enlarged; six whorls. Adult stage.

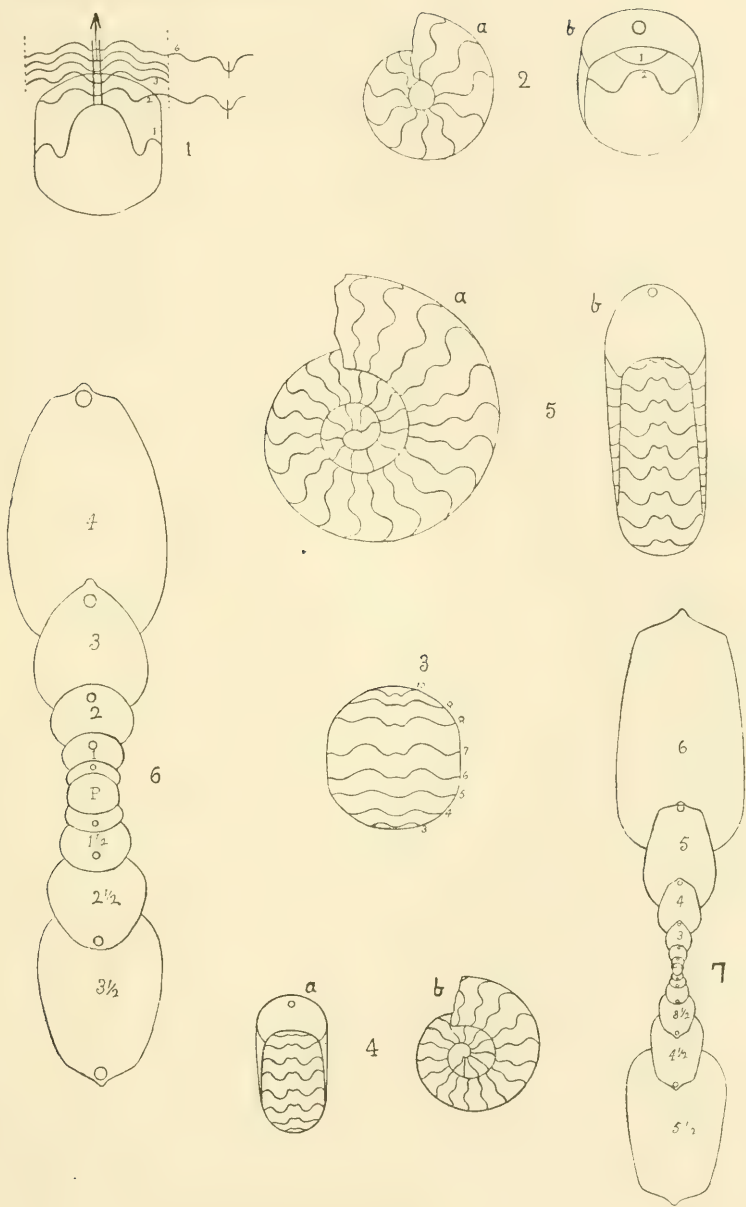
















## EXPLANATION OF PLATE D.

*Schloenbachia oregonensis* Anderson.*Development of the septa.*

FIG. 1. Septum at seven-eighths whorl; diameter 0.75 mm.; glyphioceran stage; paranepionic.  $\frac{4^0}{1}$ .

FIG. 2. Diameter 1.70 mm.; gastrioceran stage; two whorls; paranepionic.  $\frac{4^0}{1}$ .

FIG. 3. Diameter 2.50 mm.; paralegoceran stage; two and one-half whorls; paranepionic.  $\frac{4^0}{1}$ .

FIG. 4. Diameter 3.00 mm.; *Styrites* stage; two and seven-eighths whorls; ananeanic.  $\frac{4^0}{1}$ .

FIG. 5. Diameter 3.80 mm.; three and one-eighth whorls; *Parastyrites* stage.  $\frac{2^0}{1}$ .

FIG. 6. Diameter 4.86 mm.; three and one-half whorls; neanic.  $\frac{2^0}{1}$ .























## EXPLANATION OF PLATE E.

*Schloenbachia oregonensis* Anderson.*Development of the septa.*FIG. 1. Diameter 6.00 mm.; about four whorls; metaneanic.  $\frac{1}{1}^5$ .FIG. 2. Diameter 6.40 mm.; metaneanic.  $\frac{1}{1}^5$ .FIG. 3. Diameter 8.00 mm.; paraneanic; four and one-half whorls.  $\frac{1}{1}^5$ .FIG. 4. Diameter 9.20 mm.; paraneanic; four and five-eighths whorls.  $\frac{1}{1}^5$ .FIG. 5. Diameter 18.50 mm.; metephebic, early adult; about five and three-quarters whorls.  $\frac{7}{4}$ .FIG. 6. Diameter 5.60 mm.; three and three-quarters whorls; front view of Fig. 7, on Pl. IV.  $\frac{7}{4}$ .FIG. 7. *Glyphioceras* (*Muensteroceras*) *oweni* Hall. *Pal. N.Y.*, vol. v. Part II. Pl. 73, Fig. 6, for comparison with the young stage of *Schloenbachia oregonensis*.  $\frac{1}{2}$ .FIG. 8. *Glyphioceras* (*Muensteroceras*) *oweni* Hall. Loc. cit., Fig. 3, adult,  $\frac{1}{2}$ , for comparison.



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## STOLONIZATION IN AUTOLYTUS VARIANS.

P. CALVIN MENSCH.

THE development of the stolons in the chain-forming species of Syllidians has been referred to as early as 1788 by O. Franz Müller (1), and later by de Quatrefages (2), Milne-Edwards (3), Claparède (4), Von Marenzeller (5), and Langerhans (6); but it is only in the recent works of de St.-Joseph (7), Pruvot (8), and more recently in the excellent monograph of Malaquin (9), that detailed descriptions of this process are presented. In this paper I shall give results of my observations in a species which presents some characters different from those described in the previous works.

I desire to express my gratitude to Dr. M. M. Metcalf, at whose suggestion I undertook this work, also to Professor Whitman, director of the Marine Biological Laboratory at Woods Holl, Mass., for valuable assistance and advice, and finally to Dr. E. A. Andrews for suggestions as to methods of collecting the species.

*Material.*—The material for the work was collected at Woods Holl during the summer of 1895. Individuals of *Autolytus varians* in the process of budding may be found at any time of the year among the hydroids growing on the piles or in the

dredgings in and about Vineyard Sound. They occur most abundantly among the stems of *Parypha*, and during certain seasons of the year may be obtained in all stages of development by placing the hydroids in vessels of water and allowing them to remain until the water begins to become stagnant, when the specimens will collect at the surface or sides of the vessel. The mature sexual individuals are best collected in the tow-net at night in the region of piles, though they may also be found among the hydroid stems.

The abundance of the stolon-bearing forms is very much dependent upon the condition of *Parypha*, being most plentiful when the hydroids are fully developed, and disappearing almost altogether at the time when the hydroids die down. Occasional specimens in different stages of stolonization may also be found among the stems of *Bugula* and certain algae, but never in any number, even in localities otherwise favorable to their existence. In dredgings of loose sand, taken from a depth of from five to ten fathoms, quite a number of specimens were also obtained.

Associated with this species may be found two other species of the tribe *Autolytus*: the one, *A. cornutus*, a small form first described by A. Agassiz (10), which occurs in abundance on the stems of *Eudendron* and *Penaria* in the early part of the summer, before the appearance of *A. varians*; the other, *Procerea ornatus* (Verrill), very much larger, and appearing in numbers among the stems of *Eudendron* and *Parypha* later in the summer, after *A. varians* has become less abundant. In both of these species the phenomena of stolonization are essentially different from that of *A. varians*. Individual specimens of these three species may be found at any season of the year in process of budding.

*Methods.* — For surface study living specimens were used almost exclusively, the distortions produced by the killing fluids frequently being so marked as to make some of the most important details uncertain. By allowing the animals to remain in a small quantity of sea water until it becomes somewhat stagnant, they become sufficiently inactive to permit the employment of pressure and considerable manipulation without producing



unfavorable contractions. Very dilute solutions of methylene blue have been of service in studying some of the chains of living individuals, the stain soon becoming sufficiently deep to be of service without producing any noticeable irritation.

For sectioning, on account of the small size of the animal, great care is necessary in killing the specimens, so as to avoid contortions and separation of the stolons. Several methods were employed. One was to place the worm in very dilute alcohol (3-5%), and in the course of several hours to gradually increase its strength until the animal has become thoroughly benumbed, after which it was placed into the killing fluid. This method gave good results in many cases, but frequently the process of narcotizing required so much time that the sections were ruined. Another method which gave good results was to plunge the worm into 60% alcohol, remove at once into fresh sea water, and subsequently add alcohol until it was thoroughly stupefied, after which it was placed into the fixing fluid. The best method for killing the animal extended, whenever other fixing fluids than 70% alcohol were employed, was found to be by placing it on a slide and drawing off most of the water, then applying a very weak solution of the fluid by means of a small brush and gradually increasing the strength of the fluid. In this way frequently almost perfectly straight chains were obtained.

A number of fixing fluids were tried. Those which gave the most satisfactory results were Perenyi's fluid, corrosive sublimate, picro-sulphuric with corrosive, Flemming's stronger and weaker solutions, and 70% alcohol, the latter giving for general study uniformly the best results. For staining, borax carmine and haematoxylin were used.

#### DESCRIPTION OF THE SPECIES.

Two distinct varieties of *A. varians*, described and named by Verrill (11), may be found among the stems of *Parypha*, both of which occur in about equal numbers. The larger variety is from 10 to 20 mm. in length, is flesh colored, and, when examined under a medium magnification, may be seen to contain a

large number of red spots extending along the walls of the alimentary canal through its entire length, but particularly numerous in the region of the oesophagus and in the posterior and more mature stolons. The free-swimming stolons of this variety have a distinctly light red color and are considerably larger than those of the other variety. The other variety has a greenish hue, with few or no red spots along the alimentary canal, and is somewhat smaller and more slender. Intermediate forms are, however, frequently found, and the distinction between the two varieties is not so well marked in the parent stock as it is in the mature stolons. The free-swimming stolons of this variety, besides being smaller in size, are light green in color and somewhat iridescent. They differ very little in size from the free-swimming stolons of *A. cornutus*, but the male stolons can readily be distinguished from these by the fact that the *Polybostricus* of *A. varians* has swimming setae wanting on the three anterior parapodia, while in *A. cornutus* they are wanting on the six anterior pairs. The *Sacconereis* of either species can also be readily distinguished by the characters common to each mode of stolonization.

The *parent stock* of *Autolytus varians* (Pl. XIII, Fig. 1) consists of a series of setigerous segments varying in number from nineteen to as many as fifty-eight, the larger and older individuals always containing the larger number of segments. Anterior to the first setigerous segment is a segment (Pl. XIII, Fig. 2, *b.s.*) called the buccal or tentacular segment, in which parapodia are absent; but instead of these are present two pairs of cirri, called the dorsal (*d.t.*) and ventral (*v.t.*) tentacular cirri respectively. The dorsal tentacular cirri are longer and considerably thicker than the ventral pair, which are short and slender and more like the dorsal cirri of a setigerous segment. The segment itself is usually considerably narrower than the succeeding setigerous segment. Anterior to the buccal segment is the head, which consists in this species of a rounded lobe with two pairs of eyes, an anterior larger and posterior smaller pair, bearing a single dorsal median (*d.m.*) and a pair of lateral tentacles (*l.*). A pair of rudimentary palps form the ventral appendages of this lobe.











Attached to the last segment of the parent stock in mature individuals may be found a chain of stolons (Pl. XIII, Figs. 1 and 6) in different stages of development. Of these stolons the posterior is the oldest and most matured individual; the one next to it being somewhat younger and less mature, and the ones anterior to this being progressively younger and less mature, so that the most anterior presents only very faintly the outline of a stolon. The number of such stolons in a single chain may vary from several to as many as eight, and appears to be dependent upon the size of the parent stock and the sex of the stolons. In a larger chain, where the number of stolons may be as many as seven or eight, the different stages of development are progressively represented in each successive stolon. In a chain of fewer stolons, however, this is not so marked, and more frequently chains composed of but several stolons are found in which an almost mature posterior stolon may be attached to a very young and immature anterior stolon. Frequently specimens of this species may also be found which contain but a single, often quite mature, stolon, and give no evidence of a chain formation. Such specimens are usually smaller and younger in appearance and seem to indicate the very beginning of the process of stolonization.

Anterior to the youngest stolon and forming the connective between the chain of stolons and the parent stock are a number of segments (Pl. XIII, Fig. 6, *r.e.*) which are still younger and give little evidence of belonging to a distinct stolon. This region, since it is composed of the youngest and least developed segments, I shall designate as the *embryonic region*. The segments of this region are successively produced as outgrowths from the last segment of the parent stock, which segment, since it presents internal structures relative to this outgrowth that are different from those of the preceding segments, I shall refer to as the *segment of proliferation*.

#### *Description of the Free Stolons.*

Before proceeding to trace the external development of the stolon, it will be well to describe the appearance of the mature

separated stolons, or the so-called sexual individuals, *Polybostricus* (♂) and *Sacconereis* (♀). The *Polybostricus* of the red variety (Pl. XIII, Fig. 3) is about 5 mm. long; that of the green variety, 4 mm. and more slender. (This and the color being the only marks of distinction between the two varieties, I shall make no reference to either variety in further descriptions.) It consists of from eighteen to twenty-four setigerous segments, the first three of which have short parapodia, with short setae similar to those borne by the parapodia of the parent stock (Pl. XIII, Figs. 1*a*. and 1*b*.), and contain the sexual products. All the segments posterior to these, with the exception of the last or anal segment, have large elongated parapodia (Pl. XIII, Fig. 4), consisting of a ventral less muscular portion ending in a short process (*v.r.*) and bearing the short setae, and a more dorsal thickened and very muscular portion ending in a process (*d.r.*) which contains a tuft of long swimming setae. Dorsal to the thick musculature belonging to the dorsal ramus is a thin plate-like structure which forms the dorsal outline of the parapodium and constitutes the basal portion of the dorsal cirrus (*d.c.*). When the stolon is at rest the parapodia are always directed backward and pressed close against one another, thus giving this part of the body a very compact appearance and obscuring the outline of the segments. The size and direction of these parapodia as compared with the size and direction of the three anterior pairs produce a contrast sufficient to divide the stolon into two well-marked regions. At the junction of these regions the body-wall, consisting of the posterior part of the third and the anterior part of the fourth setigerous segments, shows quite prominently, and on examination appears less firm, and the line of demarcation between the segments is much fainter than it is in the parent stock. The anal segment is small, considerably narrower than the preceding segments, and in place of parapodia a pair of long, slender caudal cirri form the only appendages of the segment.

The buccal segment is well marked and bears a dorsal and a ventral pair of tentacular cirri. The dorsal tentacular cirri (*d.t.*) are very stout at the base and, when fully extended, reach as far back as the thirteenth or fourteenth setigerous segments.

The ventral tentacular cirri (*v.t.*) are much more slender and about one-fourth as long as the dorsal pair.

The head differs in shape from that of the parent stock in being broad and emarginate in front. Two pairs of eyes are present; those corresponding to the anterior eyes of the parent stock, being the larger, are placed on the ventral side of the head, so that they are not seen in a dorsal view of the animal. The ventral eyes are considerably larger than the corresponding ones of the parent stock, and bear conspicuous lenses which are directed down and outward. The second pair are smaller in size and are placed dorsally and directly over the ventral pair. Small lenses are present and directed upward and forward. A single median tentacle and two pairs of lateral tentacles comprise the appendages of the head, distinct palps being absent. The median tentacle (*d.m.*) is about equal in length to the dorsal tentacular cirrus, but is less stout at the base and is always directed backward. The anterior lateral tentacles (*a.l.*) are flattened dorso-ventrally, are very broad at the base, gradually tapering as they curve outward, and end in bifurcated processes which are not unlike dorsal cirri. The position and form of this tentacle have led Malaquin and several other investigators to regard it as being formed by the fusion of the palp with the lateral tentacle, the inner ramus representing the palp, the outer, the anterior lateral tentacle. The posterior lateral tentacles (*p.l.*) are short and straight, inserted anterior to the dorsal eyes and usually directed forward, reaching a little beyond the margin of the head. This pair of tentacles is not represented in the parent stock. The mouth opening of the *Polybostricus*, as also of the *Sacconereis*, lies a little anterior to the base of the ventral tentacular cirri and is directed downward.

The *Sacconereis* of this species (Pl. XIII, Fig. 5) is from 3 to 4 mm. in length and contains from sixteen to twenty setigerous segments. Swimming setae are usually absent from the first two setigerous segments, sometimes only from the first.

The buccal segment is narrow dorsally; ventrally, however, it is quite well marked. The dorsal tentacular cirrus is absent, but a small papilla (*d.t.*) occupying the same position may be

found in most of the specimens, and may be regarded as the rudimentary representative of this cirrus.

The head is less emarginate in front than that of the *Polybostricus*, has a median (*d.m.*), and but a single pair of lateral tentacles (*a.l.*), and is very similar in appearance to the head of the parent stock. Palps and the posterior lateral tentacles are absent, and the anterior lateral tentacles are not thickened and bifurcated, as in the male stolon.

The fully matured *Sacsonereis* carries on its ventral side an egg-sac (*o.v.*) filled with eggs. This sac consists of a thin membrane attached to and formed from the tissue of the under surface of segments 4 to 8 or 9. The anterior part of the body as far as, and sometimes including, the fourth setigerous segment is free, as are also the segments posterior to 8 and 9. When the animal is at rest the free portions of the body are usually coiled around the egg-sac in a spiral manner, in this way forming a protection for the delicate sac. When in motion, however, the free regions of the body are fully extended and the egg-sac bulges considerably both ventrally and laterally. The color of the less mature eggs is bright red; but as they become more fully matured they assume a darker and almost black appearance.

In comparing the head structure of the mature male and female stolons with those of the parent stock it is evident that, with the exception of the fusion of the palps with the anterior lateral tentacles, the presence of the posterior lateral tentacles in the male, the absence of palps and dorsal tentacular cirri in the female, and the shifted position of the anterior eyes, the head parts of the stolon are an almost exact reproduction of the head parts of the parent stock.

The movements of these free stolons as compared with those of the parent stock are very active, and notwithstanding the size of the egg-sac, the mature *Sacsonereis* is able to move about with great agility.

*Sexual Characteristics of the Chain of Stolons.*—The chain of stolons is always unisexual, all stolons of a chain being either male or female. The sex of the chain can, even in very young stolons, be distinguished by the bifurcation, in the male,



of the anterior lateral tentacle, which becomes evident soon after the tentacle has made its appearance.

The male and female chains of *Autolytus varians* also show another difference very similar to that observed by Malaquin (9), de St.-Joseph (7), and Pruvot (8), in other chain-forming species, *viz.*: the male chain is always longer and contains the greater number of stolons. Few female chains of this species are found with more than four or five stolons, while male chains may have as many as eight stolons.

The relative number of male chains appears also to be greater than that of the female chains. This fact is particularly evident among the free individuals, where five or six males may be found to a single female.

#### EXTERNAL PHENOMENA OF STOLONIZATION AS REPRESENTED IN THE CHAIN OF STOLONS.

The successive stages in the development of stolons of a chain are well shown in a male chain, as is represented in Pl. XIII, Fig. 6. This chain consists of six well-defined stolons, with a distinct embryonic region (*r.e.*), and is attached to the thirty-fifth segment of the parent stock. Externally the thirty-fifth segment, which is in this specimen the segment of proliferation, presents no characters different from those of the preceding setigerous segments.

The embryonic region of this specimen consists of five segments, the two anterior of which are of equal size, and, being the youngest segments of the series, may be regarded as typical embryonic segments. These two segments are very much smaller than the segments of the parent stock and show no signs of parapodia. The third, fourth, and fifth segments are slightly more advanced and present on each side small papilla which represent rudimentary parapodia. These segments are of equal size and differ sufficiently from the two segments preceding them and the series following to be regarded as having been formed in close succession and as developing at an equal rate toward the formation of a new stolon (St. A). Very frequently instead of three equally developed segments, as repre-

sented in this figure, four such segments are present. In all cases, however, whether three or four segments be present, the first and the last are a little larger and more developed than the intervening segments.

The beginning formation of this stolon is more evident in a slightly older series of this kind, with four segments, as is represented in Pl. XIII, Fig. 7. Segments 1, 2, and 3 represent the three appendage-bearing segments of Fig. 6 (St. A). Segments 1 and 3 have increased in size and bear lateral appendages of about equal size, while segment 2 has increased less and has smaller appendages. Between segments 2 and 3 a new segment has made its appearance and presents the same embryonic characters as do segments 1 and 2 in the embryonic region of Fig. 6.

In such a series as is represented in Fig. 7 there are present all the segments necessary for the formation of the different regions of a mature stolon. Of these, segment 1 becomes the first setigerous segment from which will be developed the head and the buccal segment; segment 3 forms the anal segment; and segment  $x$  represents the region of new growth which contributes to the elongation of the stolon. The origin of segment  $x$ , as we shall see in the study of a section of this region, appears to be from the anal segment and is not dependent upon any contribution from segment 2 in the process of its development. In this way segments 1 and 3 become the important factors in the development of the stolons, while segment 2 remains as an indifferent zone which is constantly being increased by the addition of new segments posteriorly. Malaquin (9) in describing the development of the stolon in Myrianidae lays great weight upon the importance of the anal segment (Pygidium) so far as its early appearance is concerned, but pays less regard to the part it plays in the formation of the embryonic segment ( $x$ ) of this stolon, a fact which I regard as of no little importance.

The origin of this stolon would then be by (1) the successive outgrowth from the last segment of the parent stock, or segment of proliferation, of three embryonic segments similar to the three segments of stolon A in Fig. 6, followed by (2) the



addition of a fourth segment ( $x$ ), while at the same time lateral outgrowths are appearing on segments 1 and 3 and a little later on segment 2, thus presenting a stage as represented in Fig. 7.

Two regions of growth may accordingly be distinguished as having been active in this early stage of development. The first region is that posterior to and including the segment of proliferation and has contributed three, or, in some specimens, four segments to the formation of the stolon, these segments forming the most anterior setigerous, the anal, and one or two indifferent segments. Of these segments the anal is the oldest, the others being successively younger from this segment forward, while the first setigerous is, therefore, the youngest. The second region of growth is anterior to and includes the anal segment, and supplies new segments for the lengthening of the individuals. This region in *Autolytus varians* does not contribute to stolon A in Fig. 6, but makes its appearance at a little later stage, thus not becoming active until after the outline of the stolon has been clearly defined.

Posterior to this zone of embryonic segments in Fig. 6 is the most anterior stolon (St. 1), which represents in a rudimentary condition all the regions of a mature stolon. This young stolon consists of eight well-marked segments, the anterior ones being the larger, while the posterior ones are considerably smaller. The first four segments have rudimentary parapodia, with small setae just appearing, and distinct dorsal cirri. The fifth segment has still more rudimentary appendages, similar to those of stolon A. The sixth and seventh segments are the most embryonic and show no evidence of lateral appendages, but present an appearance like the embryonic segments in the anterior part of the chain. The eighth or anal segment has a pair of caudal cirri (Fig. 8, *c.c.*) of considerable length, the development being sufficiently advanced to bear evidence of the early origin of this segment.

On the anterior dorsal half of the first segment of this stolon a new region of growth ( $c.$ ) has made its appearance. This is the first indication of the head and consists, in a stolon of this age, of merely a thickening of the tissue in the anterior half of

the first setigerous segment and its forward extension slightly beyond the anterior region of this segment. The forward extension of this new tissue is confined in greater part to the dorsal surface and has the appearance of a thickened plate, with the lateral portions bulging out so as to give the appearance of the two lateral lobes. At the sides this plate tapers into a narrow rim which is lost in the lower lateral margin of the segment on a plane with the parapodia. This stage of development has been reached by a gradual elevation and forward extension of mostly the anterior part of the first setigerous segment. The very first indication of this growth is a slight thickening of almost the whole dorsal wall of the segment, which gradually becomes more marked anteriorly and soon forms a prominent ridge over the anterior dorsal and lateral portions of the segment. Subsequently this ridge begins to bulge forward in the form of a prominent rim, with two faint lobes laterally, beyond which it gradually becomes less marked until near the ventral surface, where it disappears.

Stolon 1, therefore, presents two regions of growth. The one anterior to and including the anal segment which, as I have already indicated in the description of the preceding stolon, is rapidly contributing to the increase of the number of setigerous segments. The other is a new region, which has appeared prominently for the first time in this stolon. This new region is developed secondarily in a segment which has originally been separated from the parent stock, and appears shortly after the region of growth anterior to the anal segment has become active. Thus the different structures of this stolon have originated in three ways: (1) from segments derived directly from the parent stock; (2) from segments derived from the anal region of growth; (3) from outgrowth from the anterior segment of the series. Of these, the last two contribute to the future development of the stolon.

Stolon 2 represents a stage a little more advanced than stolon 1, and presents the most important changes which next take place in the process of development. It consists of fifteen setigerous segments, the anterior ones of which are considerably larger than those of stolon 1, while the more posterior com-

pare well in size with those of the preceding stolon, thus making the outline of the stolon appear somewhat wedge-shaped. The parapodia and dorsal cirri in the anterior half of the body are well developed, while those in the posterior half are progressively smaller from before backward as far as the pre-anal segment, which segment, as in the preceding stolon, represents the region of youngest growth. In this and the two preceding segments, in a progressively less degree however, the parapodia are quite rudimentary, and the segments present an appearance very similar to the parapodia-bearing segments of stolon A. The anal segment has also increased in size, and the caudal cirri have been considerably elongated. In this figure the segment is almost completely hidden by the head of the succeeding stolon.

In comparing the setigerous segments of this stolon with those of the preceding, it will be noticed that parapodia, either well developed or rudimentary, are present on all the segments of the stolon except the anal. This would indicate that the purely embryonic growth, as represented in the embryonic segments of the preceding stolon, had passed its stage of greatest activity and is not as prominent as in the preceding stolon, the stolon having already attained a considerable length and the addition of new segments from this stage on progressing very much slower.

The head of this stolon has also advanced considerably, both in size and in the development of new structure. Its antero-posterior diameter has increased sufficiently to equal that of one of the most advanced setigerous segments of the stolon. The lobed structures, appearing in the preceding stolon and becoming more prominent in intermediate stages, have given rise to the rudimentary anterior lateral tentacles (*a.l.*). These tentacles are thick and knob-like in structure and are directed forward and outward. The dorsal median tentacle (*d.m.*) has also appeared in the form of a slender tentacle, extending as far back as the second setigerous segment. Two eyes (*e.a.*) have appeared, and have in this individual already attained a considerable size. These are located at the base of the lateral tentacles, and hence correspond to the anterior eyes of the

parent stock. The first indication of these eyes is marked by the appearance of minute pigment spots in a stage intermediate between this and stolon 1, at a time when the lateral and dorsal median tentacles have become about one-half as large as represented in stolon 2.

A lateral view of the developing head of this stolon is represented in Pl. XIII, Fig. 8. In this view it will be seen that the zone of new growth extends forward considerably more than in the preceding stolon, and also, that instead of extending down laterally as far only as the parapodia, it now extends to the ventral median line. The whole structure at this stage assumes the appearance of a separate segment, being narrower above and narrowing down laterally until on the ventral surface it forms a very narrow strip of tissues, the outlines of which are lost as it approaches the median line. On the lateral surface of this new growth, a little dorsal to the insertion of the dorsal cirri, a small papilla (*d.t.*) has made its appearance. This bud represents the rudimentary dorsal tentacular cirrus, and first appears at about this stage of development, being somewhat later than the dorsal median tentacles, which may be found in stolons of a stage of development intermediate between this and stolon 1. In a stolon of this age, then, there are present: (1) a pair of anterior lateral tentacles; (2) the dorsal median tentacle; and (3) the pair of dorsal tentacular cirri, all having appeared successively in the order enumerated. Since the tentacular cirri form a part of the buccal segment, it may be assumed that already in this stage this new tissue is being differentiated into the buccal and head segments, even though there are no external indications of this division.

Between the anal segment of the preceding stolon and the head parts of stolon 2 (Pl. XIII, Fig. 8) there exists a narrow structure which belongs, properly speaking, to neither of these segments, and which I shall designate as the *region of separation* (*r.s.*). This region forms the connection between the two stolons, and in earlier stages seems to belong to and constitute an undifferentiated part of the anal segment. At a stage represented by these two stolons, however, it seems, externally at least, to be entirely distinct from the anal segment, and presents



the appearance of a very narrow lighter band of interposed embryonic tissue. It is in this tissue that the separation of the stolon from the chain will take place. It becomes plainly visible for the first time between consecutive stolons of this stage of development, and appears most prominently here, being in later stages hidden by the overgrowing head of the succeeding stolon, and also probably because of the structural changes, to which I shall refer later.

Stolon 3 shows a few more advances in the process of development which make their appearance in an individual of this size. This stolon consists of eighteen setigerous segments, followed by the anal segment, not shown in this sketch. The anterior segments are somewhat larger than those of the preceding stolon, and the parapodia and dorsal cirri are more prominent and more fully developed, the whole individual presenting a larger and more mature appearance. The breadth of the head has increased in conformity with the breadth of the first setigerous segment, and a second pair of eyes (*e.p.*) has appeared, being placed posterior to the first pair and in a line with the insertion of the dorsal median tentacle. These eyes are not so widely separated from one another as are the first pair and are considerably smaller. The arrangements of the eyes on the head of this stolon very closely simulate the position of the eyes on the head of the parent stock, although the shape of the head is decidedly different. The appendages of the head have also increased in size; the dorsal median tentacle has increased by about the width of two segments and reaches as far back as the third setigerous segment. The two anterior lateral tentacles, besides having increased in length, also show indications of branching by the appearance of a small bud on the median side of each tentacle. The bifid tentacle being characteristic of a male stolon, it is possible even at this stage of development to distinguish the sex of the stolon and hence of the chain. In a dorsal view of this stolon the outline of the buccal segment is not more visible than in the preceding stolon, and were it not for the projection of the dorsal tentacular cirri (*d.t.*) evidence of the existence of this region would be wanting. Laterally the buccal segment has attained a consid-

erable dimension, particularly in the region of the dorsal tentacular cirrus, and its outline is more easily defined than in the preceding stolon. More dorsally its outlines, as in the preceding stolon, are lost in the external undifferentiated tissue which constitutes what is here designated as the head.

While the dorsal and lateral surfaces of the chain thus far examined have undergone numerous changes by way of the addition of new tissue and the formation of new structures, the contour of the ventral surface has remained very much less disturbed, and a ventral view of parts of stolons 2 and 3 (Pl. XIII, Fig. 9), in which dorsally great changes have taken place, indicates comparatively little activity in the surface of this region, the changes being confined to the addition of new segments of similar character and not to the ventralward extension of dorsal thickenings. Such a view of this part of the chain would, therefore, present a comparatively smooth surface ventrally, with marked lateral irregularities caused by the difference in size of the various segments. In this figure may be distinguished the exact outline of the anal segment (*a.s.*) of stolon 2, the true size of its caudal cirri, and the position of this segment in relation to the head of the successive stolon. The zone of separation (*r.s.*) appears as a very narrow band of tissue, the outlines of which are plainly visible in this view.

Stolon 4 presents a still later stage in the process of development. It consists of twenty distinct setigerous segments, the last four or five of which are quite narrow as compared with the preceding, and thus gives the outline of this stolon an appearance quite different from that of the preceding ones and makes it conform more closely to the outline of the free *Polypostricus*. As compared with the preceding stolon, the segments of the anterior two-thirds of this stolon have increased in breadth and a greater number of parapodia in an advanced stage of development have become visible. The appearance of one of the more advanced parapodia of a stolon of this age is represented in Pl. XIII, Fig. 10. At this stage the ventral branch of the parapodium (*v.r.*) has attained its full development and is not unlike the lateral appendages which constitute the entire parapodium in the parent stock so far as the position and num-



ber of setae are concerned, and differs but little in size. The dorsal cirrus (*d.c.*) is, however, placed a little more dorsally than that of the parent stock, and between the dorsal cirrus and this ventral branch of the parapodium a new growth of tissue (*d.r.*) is arising. This new growth is the rudiment of the dorsal branch of the parapodium and becomes visible for the first time in a stolon of this stage of development.

In a dorsal view the appearance of the head has remained unchanged, except that the anterior eyes have approached more closely the lateral margin of the head. The shifting in position of the anterior eyes at this stage is very slight, but still sufficient to mark the beginning of important changes that are about to make their appearance in stolons of this size. These changes consist in the gradual outward shifting of the anterior eyes, in process of which they are first carried laterally, then ventrally, until finally in the free-swimming individual they occupy a position directly ventral to the posterior eyes. The shifting, as we will see later in the study of transverse sections of this region, is due to the large increase in the number of nerve cells in the middle region of the head, the region occupied by the eyes being in consequence carried successively lateral and ventralward. In a lateral view of a stolon a little more mature (Pl. XIII, Fig. 11), the anterior eyes (*e.a.*) occupy a lateral position, and the changes in the contour of the head have been such as to bring the anterior and the posterior eyes nearer a transverse line with one another. The appendages of the head of stolon 4 have increased considerably in size, the dorsal median tentacle reaching as far back as the sixth setigerous segment, while the anterior lateral, besides elongating, have also increased in thickness and are bifurcated for more than one-half their length. The outlines of the buccal segment have not as yet appeared dorsally, although the dorsal tentacular cirri (*d.t.*) have attained a considerable length. In a lateral view of the slightly older stolon, represented in Fig. 11, this segment is quite well marked, and besides the presence of the now elongated dorsal tentacular cirri (*d.t.*), a rudimentary ventral tentacular cirrus (*v.t.*) has also made its appearance. In this figure may also be noticed the region of separation (*r.s.*),

which is still visible in a lateral view of a stolon of this age. On comparing it with the same region of younger stolons, it will be noticed that it has become more constricted and forms a much narrower bond of union between the two stolons, the constriction at this stage being noticeable both ventrally and laterally. It is at this stage of development also that the young stolon shows the first indication of independent movement.

Stolon 5 represents a stage in which the stolon has attained its fullest length and breadth, and in which the general contour of the *Polybostricus* is becoming more apparent. The most striking difference between this and the preceding stolon exists in the large increase in the size of the parapodia, particularly in the middle region of the body, the development of the parapodia being so great as to give the segments an entirely different appearance in a surface view. If the anterior or posterior surface of such a parapodium (Pl. XIII, Fig. 12) be examined, it will be noticed that the dorsal ramus (*d.r.*) has increased very much in length and slightly exceeds the ventral ramus both in length and breadth. The two rami remain fused in the line of junction and thus form a broad, flattened appendage with a tuft of setae protruding from the ventral angle. In the outward growth of the dorsal ramus the dorsal cirrus has been carried outward from its position on the side of the body-wall, and now occupies a position on the dorsal angle of the parapodium. As compared with the more simple parapodium of the parent stock (Pl. XIII, Fig. 1a.), this parapodium is not alone very much larger, but by the outward growth of the dorsal ramus the position of the ventral ramus has been so changed as to cause it to assume a more transverse position instead of inclining ventralward, as it does in the parent stock. Normally, when at rest these parapodia are directed slightly backward, thus already assuming the position of the parapodia of the free forms. The parapodia of the first three setigerous segments, however, do not undergo so complete a change. They remain considerably smaller, and instead of being directed backward, are placed directly at right angles to the axis of the stolon, the growth of the dorsal ramus being confined to an elongation of that region sufficient to carry the











insertion of the dorsal cirri to a position almost dorsal to that of the setae.

The head of this stolon has also assumed a different appearance, and in a dorsal view has attained the size and shape of the head of the *Polybostricus*. The anterior eyes have shifted laterally and now occupy a position very similar to that represented in Pl. XIII, Fig. 11. The posterior eyes have been carried forward to nearer the anterior margin of the head and have increased considerably in size. The appendages of the head have undergone no changes save an increase in length. The buccal segment is now visible dorsally as a narrow segment considerably less in transverse diameter than the head and very indistinctly separated from it. The dorsal tentacular cirrus has increased very much in length, while the basal portion has increased to a thickness greater than the dorsal width of the buccal segment. The ventral tentacular cirri have attained a length about equal to that of a dorsal cirrus.

Stolon 6 represents the stage of advancement shortly before the stolon is separated from the chain. As compared with the preceding stolon the most striking change is the still greater increase in the size of the parapodia and the presence of swimming setae in all the parapodia except the three anterior. The increase of the parapodia in thickness as well as in length has given the posterior three-fourths of the stolon a more compact appearance, thus making the distinction between the three anterior and the posterior segments very much greater. The larger parapodia are directed backward still farther, and in this way a considerable space is left between the third and fourth pair of parapodia. In an anterior view of such a parapodium it will be seen that the different parts of this appendage have assumed the appearance of those of a parapodium of a free-swimming individual; the dorsal ramus extending somewhat beyond the ventral ramus and containing at its extremity a tuft of long, slender swimming setae; the dorsal cirrus inserted some distance in from the insertion of the setae; the ventral ramus ending in a small tubercle containing a tuft of setae; while posterior to this tubercle the two rami unite to form a broad plate made up by the musculature of this appendage.

In the head the anterior eyes have shifted more ventrally and now lie almost beneath the posterior pair. A new pair of tentacles, the posterior lateral (*p.l.*), have made their appearance simultaneous with the appearance of the swimming setae. These are inserted anterior to the dorsal eyes and consist of bud-like processes that extend a little beyond the margin of the head. The buccal segment has become more prominent and can be more readily distinguished from the region constituting the head. In a lateral view of the anterior part of this and the lateral portions of the preceding stolons, it would be noticed that the ventral surface of the anal segments of the preceding stolon no longer lies in the same plane, but that the buccal segment and even ventral parts of the succeeding segment are elevated considerably above the plane of the anal segment, thus causing the attachment of this stolon to the preceding one to appear to be more on the dorsal surface of the anal segment than posterior, as in the preceding unions. This is made very evident in specimens that are in a state of partial contraction, but is also well marked in the normal state and is due to the increase in size of the head, which, thickening only dorsally, pushes ventralward the tissues constituting the zone of separation.

After this stage of development has been attained the independent movements of the stolon become very active and separation from the chain soon takes place. The separation of the stolon takes place in the region of separation previously referred to and which already prior to this stage, as will be seen in the examination of sections of this region, has undergone a ventral degeneration by which this structure is gradually weakened so that when the stolon becomes more active, rupture of the tissue is easily effected.

The stolon when liberated from the chain has attained the size of a mature free-swimming stolon and possesses all the appendages, most of which have attained their full size and undergo but few external changes in becoming completely mature. The most important change concerns the carrying of the anterior eyes exactly ventral to the posterior pair. This process seems to be completed immediately after the separation

of the stolon and is effected by the contraction of the ruptured tissue and a consequent drawing down of the ventral tissues of the head. The shifting of the head tissues has a slightly rotating effect upon the anterior lateral tentacles so that the plane of bifurcation, instead of being horizontal, is more vertical. At the same time the tentacles are bent outward and become still more thickened at the base. The posterior lateral tentacles also increase in length and are extended forward some distance beyond the margin of the head. As the spermatozoa which are contained in segments 1, 2, and 3 ripen, the walls of these segments become somewhat distended and the outlines of the segments remain quite definite. At the same time the walls of the segments posterior to these more or less lose their outline, and it is with difficulty at times that the outlines of the segments can be distinguished at all save by the location of the parapodia. This condition marks the complete maturity of the sexual products and hence also of the stolon, and may appear shortly or some time after its separation from the chain.

*Development of the Female Stolon.* — The development of the stolons of a female chain presents external changes very similar to the development of those comprising a male chain. Since the female chains contain fewer stolons, the different stages in the progress of development are not so well marked in the same chain, and the chain usually is composed of successive stolons which exhibit greater differences in age than in a male chain; the shortness of the chain being due, therefore, not to an earlier separation of the stolon, but to a greater lapse of time between the periods of stolon formation at the beginning of the process. The early stages of the processes are identical with those in the male chain, except for the bifurcation of the anterior lateral tentacle and, in the older stolons, the appearance of the dorsal tentacular cirri. The ventral tentacular cirri appear for the first time in a stolon which has reached a stage of development corresponding to that of stolon 4, Pl. XIII, Fig. 6. In a stolon of this age the parapodia of the female stolon are not unlike those of the male. At a somewhat later stage, however, in a stolon corresponding in age to stolon 6, they present an appearance quite different from those of the male

(Pl. XIII, Fig. 13). In comparing it with a parapodium of the *Polybostricus*, it will be noticed that, besides being smaller, the dorsal ramus (*d.r.*) has remained quite short, while the ventral has pushed out some distance beyond it, thus carrying the insertion of the short setae external to that of the swimming setae and presenting a condition reverse to that of the male parapodium. The insertion of the parapodium does not extend as far dorsal as it does in male stolons, and hence the dorsal contour of the stolon remains more like that of the parent stock. As no further growth takes place after this stage of development has been reached, such a parapodium presents all the characteristics of a parapodium of a fully matured *Sacconereis*. The sexual products appear very early, and even in stolons no older than stolon 1, Fig. 6, small ova are plainly visible through the thin body-wall, and by the time the stolon reaches a stage corresponding to that of stolon 6, it assumes a darker color and becomes very much more opaque, due to the accumulation of a large number of ova in the body cavity, the outlines of which can be distinctly seen in a surface view.

The separation of the female stolon takes place some time before the formation of the egg-sac, and at the time when the stolon breaks from the chain it is well distended with closely packed ova which extend even into the cavities of the parapodia, and the swimming setae have been fully developed. Such specimens at once acquire the habits of the fully matured individual and may be found swimming at the surface associated with the fully matured individuals. Shortly after the liberation of the stolon the ventral cuticle begins to become more distended, and before long the ova begin to accumulate in the ventral sac and the stolon presents the appearance of the mature *Sacconereis*.

#### HISTOLOGICAL PHENOMENA OF STOLONIZATION.

*Internal Structures of the Mature Stolon.*—Prior to taking up the histological changes in the development of the young stolons, I shall briefly describe such structures of the mature stolon as form the regions in which the most important



processes of stolonization take place. As the principal phenomena are those which are connected with the development of the head and buccal segment, while those concerned in the development of the remaining portion of the body present no important characteristics different from direct development among Syllidians, a section of the anterior segments of a mature stolon will present all the important structures concerned in the process of stolonization.

Pl. XIII, Fig. 14, represents a longitudinal median section through the head, buccal, and the anterior part of the first setigerous segments of a mature male stolon. The first setigerous (*sg.1*) and buccal segments (*b.sg.*) are separated by a distinct dissepiment (*dis.*). Between the buccal segment and the head the musculature of the dorsal median tentacle (*t.m.*) forms the only partition, and laterally where these fibers do not exist, the coelomic cavity of the buccal segment extends forward into the tissues of the head for a short distance, so that internally these two regions are not so distinctly separated. Dorsally, as well as laterally, the body-wall of the buccal and setigerous segments consists of an epidermis covered by a thin cuticle (*cu.*) and overlying a layer of circular and longitudinal muscle fibers. The epidermis (*ect.*) consists of a single layer of cells distinctly separated from the underlying tissues, and its outlines are clearly defined. Ventrally, however, the epidermal cells are very indistinctly outlined, and, particularly in the regions of the ganglia of the ventral cord, are so intimately associated with the ganglion cells (*c.n.*) of the ventral cord that the epidermis cannot be distinguished from the underlying tissues by means of the ordinary methods of preparation. The endodermal cells, as they approach the mouth-opening (*o*), become smaller, and where this structure joins the ectoderm the outlines of the cells are lost. The body space of the first setigerous segment is filled with sperm cells. The cavity of the buccal segment also contains sperm cells (*s.p.*), but they are usually fewer in number and less compact.

The head (*c*) is an almost solid structure, its only cavity being the space on either side of the muscles of the dorsal median tentacle, which is in reality a forward extension of the

buccal cavity and not a separate cavity. In a median section like the one represented in Pl. XIII, Fig. 14, this space is not seen, and the posterior part of the head in this plane is made up entirely of the muscular structures of the dorsal median tentacle (*t.m.*), which reach as far ventralward as the junction of the epidermis with the endoderm. Anterior to the musculature of this tentacle is the brain structure, which consists of a central brain mass surrounded by a dense mass of ganglion or brain cells (*c.n.*). The central brain mass or medullary substance consists of two distinct lobes, the anterior of which (*cb.a.*) is the smaller, and gives off a pair of nerves to the palps and sends fibers into the circumoesophageal nerve ring. The posterior lobe (*cb.p.*) is the larger, and is partly divided into two smaller lobes by a fissure that extends more than two-thirds across the lobe. It supplies the tentacles and eyes with nerves and gives off fibers which form the greater part of the circumoesophageal nerve ring. Examined under a high power, this central brain tissue is found to consist of very minute fibers interlacing one another and having no distinct direction except in regions from which nerves are given off, where they assume a more parallel course. The tissues surrounding this central brain substance consist of a mass of cells imbedded in a network of very fine fibers, not unlike the fibers of the central mass, but less densely arranged. The brain cells are not scattered uniformly around the medullary substance, but are so crowded as to form centers—these centers occurring at the origin of the nerves and at the fissures in the medullary substance. The outlines of these cells, particularly in the region of the centers, are very indistinct, and even away from these centers it is difficult, by means of the ordinary methods employed, to distinguish the exact shape of the cells. In the less dense regions of the eyes, however, as has been observed by Malaquin in other Syllidians, distinct unipolar cells may be distinguished.

The epidermis overlying this cortical substance, just as in the case in that underlying the nerve cells of the ventral cord, cannot be distinguished from the cortical substance, the cells of both being so intimately associated that the outline of the



epidermis is entirely obscured. Posterior to and over the median tentacle, as well as over the other tentacles, the epidermis is present as a distinct layer of tissue; but where the base of the tentacle encroaches upon cortical tissues the outline of this layer becomes abruptly lost, and in regions anterior to this tentacle the cortical substance seems to be composed of cells of uniform structure, and to extend from the medullary substance to the borders of the cuticle without presenting even a peripheral line of nuclei to indicate the presence of such a layer. This intimate association of cortical and epidermal cells was first pointed out by Fraipont (12) in *Protodrilus*, and it appears to be a condition common to all Syllidians. In sections of the head of *Autolytus pictus* and *Eusyllis* there exists, according to Malaquin (9), a peripheral arrangement of cells, by the orientation of which he is able to recognize an epidermal layer. Such an arrangement of cells is not sufficiently apparent to justify the recognition of a distinct epidermal layer in a section of the head of a mature stolon of *Autolytus varians*; and in a similar section of the head of the parent stock the orientation, while being more evident, is not sufficiently marked to indicate the presence of a true epidermis. What results are to be obtained in the distinguishing of these two structures by the employment of special methods, hitherto not tried, I shall endeavor to demonstrate in a later paper.

The internal structures of the head of the parent stock are so similar in character and arrangement to those of the stolon that it would be useless to repeat the figure. The medullary substance of both are alike in size and divisions into lobes, while the arrangement of ganglion cells into centers is as well marked in one as in the other, and the only way by which a section of the adult head might be distinguished from a similar section of the head of a mature stolon would be by differences in the alimentary canal, the increase in size of the median tentacle, and the absence posterior to the median tentacle of a ciliated region known in species of *Autolytus* where this is more marked as the epaulets. Thus it is evident that as far as internal structures are concerned, the head of the stolon is an exact reproduction of the head of the parent stock, and the

development of the head of the stolon, therefore, means the appearance by a different method of such structures as are present in the head of the parent stock and which have been formed in the ordinary processes of development.

*Region of Proliferation.*

The changes brought about by the phenomena of stolonization are already evident anterior to the chain of stolons in the internal structures of the last segment of the parent stock (Fig. 6, 35) in an area which I have designated as the *region of proliferation*. In a transverse section of this region (Pl. XIII, Fig. 15) it is at once apparent that structures closely associated with the processes of stolonization have made their appearance which are not present in sections of segments anterior to the segment preceding the chain. These structures consist of masses of mesodermal tissue (*m.c.*), which completely fill the coelome laterally and even extend into the cavities of the parapodia. Dorsally and ventrally the tissue is scantier, and well-defined areas of body space (*coe.*) are visible. In the anterior parts of the segment this mesodermal tissue is almost entirely absent, and a section of this region would not be at all unlike a similar section through any of the preceding segments. In all preceding segments the body cavity is spacious, and such mesodermal masses can be observed only in a segment immediately preceding a chain of stolons. Examined under a higher magnification, this tissue will be seen to consist of cells which appear embryonic in structure and are in no wise different from those constituting the mesodermal structures of embryonic segments. Associated with this tissue are the muscles of the parapodia (*m.ac.*), while dorsally and ventrally may be seen the dorsal (*m.d.*) and ventral (*m.v.*) muscle bands, all of which structures, as well as those of the dorsal (*d.v.*) and ventral (*v.v.*) blood vessels, can readily be distinguished from this tissue. In regions a little posterior to the section here represented this tissue becomes more dense, fills the entire coelome cavity, and is directly continuous with similar tissues of the succeeding segments.

In addition to the appearance of this mesodermal tissue the epidermis, particularly in the dorsal region, is somewhat denser, and the cells seem more crowded than in sections of preceding segments. Ventrally the epidermis in this section is not unlike that of preceding segments, the lateral outline of its cells being quite distinct, while in the region of the ventral cord the cells are so closely associated with the ganglion cells (*c.n.*) of this region that the two structures cannot be differentiated.

Pl. XIII, Fig. 16, represents a section through the posterior part of segment 35, Pl. XIII, Fig. 6, in a plane just anterior to the first segment of the embryonic region. In this section the mesodermal tissue (*m.e.*) so nearly fills the coelomic cavity as to leave but very small dorsal and ventral spaces (*coe.*). The epidermis (*ect.*) has also increased considerably in density, both laterally and dorsally, and the cells are no longer arranged in a simple layer, but are so compactly placed as to give the appearance in transverse section of several layers. (In this section, owing to the strong contractions produced by the fixing fluid, the thickness of the epidermis is somewhat exaggerated, particularly dorsally and on the one side.) Compared with the epidermal cells in any other segment of the parent stock, the cells of this region appear to be smaller, less regular, and present more fully the characters of newly dividing cells.

The alimentary canal (*ent.*) of this section also presents characteristics different from that in preceding segments. Its calibre is very much smaller, the constriction being already apparent in Pl. XIII, Fig. 15, and the decrease in the size of the cells is quite well marked. The abundance of nuclei in the cells would also demonstrate a rapid formation of new cells in this region.

The general appearance of the tissues of these sections would, therefore, indicate that the posterior part of this segment has undergone a complete change from that of an ordinary segment of the parent stock, and that, while its external appearance remains unchanged, its internal structure has become so modified as to convert its posterior regions into a distinct embryonic center, in which new tissue is being rapidly formed and pushed back to furnish the embryonic tissue, from

which the new segments formed in the anterior part of the chain are built up.

The gradual transformation of the tissues of this segment into embryonic tissue is well shown in a longitudinal median section of this and succeeding segments of the chain. Pl. XIII, Fig. 17, represents a longitudinal sagittal section through segment 35, the embryonic region and the anterior part of stolon 1, Fig. 6. The plane in which the section represented in Fig. 15 was taken is shown at A, while the plane of Fig. 16 is shown at B. The limit of this segment (35) is well marked anteriorly by the dissepiment (*dis.*); posteriorly, however, the internal limits of the segment are lost in the masses of embryonic mesoderm (*m.e.*), which completely fill the body cavity at the posterior extremity of this segment, and the external constrictions alone define its exact limit. The dorsal epidermis at B, and posterior to this plane, shows quite a transformation when compared with that anterior to the plane A, the cells appearing considerably narrower and possessing more the characters of embryonic ectoderm. Ventrally the character of this ectodermal tissue, as seen in the longitudinal sections, is even more distinct, and in the posterior part of this segment (*e.e.*), in a plane with the denser masses of embryonic mesoderm, the outlines of these ectodermal cells are quite apparent. The tissues in the regions belonging properly to segment 35 are composed of elongated spindle-shaped cells closely crowded together, but having a clearly defined outline, particularly at the very posterior border of this segment. More anteriorly their outlines become gradually fainter, until, in a plane a little anterior to B, they assume the appearance common to the region ventral to the nerve cord in preceding segments.

From a longitudinal section of segment 35 it is, therefore, quite evident that the posterior part of this segment has become converted into an embryonic center, in which new tissue is constantly being added to the three embryonic layers, which new tissue, in the process of formation, is carried back and forms the most anterior embryonic segments of the chain. In this way the formation of all the segments preceding stolon A, Pl. XIII, Fig. 6, can be accounted for.













*Embryonic Region.*

In this region (Pl. XIII, Fig. 6, *r.e.*) I have included in the description of the external characters of the chain the very youngest segments which have been developed from the region of proliferation, and also such older segments which have already indicated by their general appearance the positions they will occupy in the mature stolon, and in which can already be distinguished the outlines of a stolon (St. A), but all of which I regard as having been derived as outgrowths from the region of proliferation. Pl. XIII, Fig. 18, represents a transverse section through the segment immediately following segment 35, and possesses the characters presented by a very young setigerous segment just after its external outlines, as represented in Pl. XIII, Fig. 6, have become distinct. Its structures differ very little from the embryonic structures of the preceding segment, and it presents the very simplest and youngest structures which can form a distinct segment. The mesodermal tissue is quite prominent and fills the entire coelomic cavity. Dorsally and ventrally the tissue appears quite loose, but laterally two distinct denser regions may be distinguished; the cells of the dorsal region (*m.e.*') being the less compact and forming a larger area, while those of the ventral region (*m.e.*'') are more compactly arranged and are confined to a smaller area near the ventral cord. Between these two regions the tissue presents the looser appearance seen in the dorsal and ventral regions. The dorsal (*d.v.*) and ventral (*v.v.*) blood vessels can readily be distinguished from the surrounding tissue, while but a few poorly defined fibers appear to mark the position of the circular and longitudinal muscles. The ectoderm can readily be distinguished from this mass of mesodermal tissue by the size of the nuclei and the direction and appearance of the cells and muscle fibers. As in the region of proliferation, this tissue is somewhat thicker and contains many nuclei placed close together, and indicates by the numerous outlines of cells the rapid formation of new ectodermal tissue. Ventrally the nerve cord (*v.c.*) passes through this tissue in three distinct strands, all of which are separated from one another and are surrounded by cells

similar to those comprising the remainder of this tissue. The appearance of the ventral cord in this section is not unlike the appearance of the cord in sections of the parent stock passing through the region of the cord between the ganglia, except that in the parent stock these strands are never so widely separated. This section is, however, taken through a plane, in which, in a more mature segment, a ganglion is present, and the examination of successive sections of this segment will demonstrate the entire absence of a ganglion in a segment of this stage of development; neither are the nuclei surrounding the cord more densely crowded than they are in any other parts of this tissue. The entodermal cells show no important differences from those of the preceding section, which represents a zone equally active in growth.

Pl. XIV, Fig. 20, represents a section through the segment immediately following the one just described, or the second segment of the chain. The most important characteristics of a section of this segment, as compared with the preceding, appears in the greater thickening of the ectodermal tissue and the scanty appearance of the mesoderm. Neither of these layers presents structures that would indicate any difference in the age of this and the preceding segments, the tissues of both being quite alike in general arrangement and appearance. The ectoderm of this segment has increased considerably in thickness and would indicate, by the more crowded appearance of the nuclei, a region in which the cells are smaller, and hence a segment in which growth seems more active than in the preceding segment. Besides this, however, no differences can be distinguished in a transverse section. The narrowing of the band of mesoderm is confined mostly to a lateral decrease in the tissue, while dorsally and ventrally no difference can be observed from that of the preceding segment.

The structural differences of these two segments are made more apparent in the longitudinal sections of Pl. XIII, Fig. 17. The plane of Pl. XIII, Fig. 18, is represented at C; that of Pl. XIV, Fig. 20, at D. If these two segments be compared, it will be seen that in passing from plane C to plane D the dorsal ectoderm becomes rapidly thicker, and the cells appear

smaller and more numerous. Ventrally there is even a more marked difference between the cells of the two segments. In the anterior segment (Sg. 1) the outlines of the cells are quite indistinct, and in this section of the ventral ectoderm appear very little different from those of a segment of the parent stock. In the second segment, on the other hand, this ventral ectoderm presents an appearance entirely similar to the ventral ectoderm of segment 35 in the plane of B (*e.e.*); the outlines of the spindle-shaped cells (*e.e.p.*) are quite distinct, and this region appears as distinctly embryonic as does the ventral ectoderm of the posterior part of segment 35.

These two segments accordingly present a stage in which the three embryonic layers have undergone very little change toward the formation of any structure of the mature segment. In the ectoderm the ventral cord forms the only distinctly differentiated structure. The cells beneath it have, in the anterior segment, assumed the appearance of the nerve cells common in this region; while in the second segment, as seen in longitudinal section, the cells have not undergone this differentiation, but still possess the characters of the purely embryonic cells of the zone of proliferation. The mesoderm of these segments, with the exception of the dorsal and ventral blood vessels and the faint bands of the muscle tissue, presents no differentiated structures, but differs in the two segments in the amount of tissue present. Both segments being embryonic in structure and still possessing characters which make them appear different from one another, if these differences are not due to the differences in age of the segments, we have presented in these two segments two distinct phases of embryonic segment formation. If these differences were due to the variance in the ages of the segments, the second segment, being the elder, would present the greater differentiation; while, as is quite evident in the longitudinal section of these regions, the anterior segment in reality presents the more differentiated structures. There must be present, therefore, already at this early stage, two distinct types of embryonic segments, each of which is destined by its internal formation to occupy a distinct position in the mature stolon. As will be seen later,

the second segment presents characters common to the last segment of each stolon, and as the segment following already shows ectodermal thickenings indicative of a head formation, this segment may be regarded as the anal segment.

Pl. XIV, Fig. 21, represents a transverse section through the posterior part of the first segment of stolon A, Pl. XIII, Fig. 6, and indicates a condition less embryonic than that presented by the two preceding segments. The ectoderm in this section is very little thickened dorsally, though in the anterior parts of this segment, as will be seen in another section, the thickening is considerable. Laterally the ectoderm has pushed out in the form of small processes which form the rudiments of the parapodia (*p.*). Ventrally the ectodermal cells have increased in number, and in the region of the nerve cord the number of nuclei (*c.n.*) has increased sufficiently to suggest a region of ganglion cells. The cord itself has increased in thickness and presents an appearance common to the ganglionic region. The mesodermal tissue has also undergone some differentiation. A coelomic cavity has made its appearance dorsally, and in part laterally, while the dorsal and ventral muscle bands (*m.d.* and *m.v.*) appear much more prominent than they do in the preceding segments. In the ventral lateral regions, however, the embryonic masses of mesoderm (*m.e.*) still exist and form quite a dense undifferentiated structure.

Sections through the second segment of stolon A show no characters different from those presented in the segment just described, save a slight decrease in the thickness of the dorsal ectoderm; while sections of the third segment of this embryonic stolon repeat almost exactly the structures presented by Pl. XIV, Fig. 20.

In a longitudinal section (Pl. XIII, Fig. 17) this stolon (St. A) appears as quite a distinct stolon. Anterior to the plane of Fig. 21 (*E*), the dorsal ectoderm of the first segment (*c.*) has become considerably thicker, and spindle-shaped cells similar to those which appeared beneath the ventral cord in the embryonic region of segment 35 are beginning to make their appearance in this region. Over the anterior part of the second segment the ectodermal cells are less spindle-shaped



and are arranged in a single layer, but posteriorly, and over the third segment, the tissue is again thickened and the cells again present a spindle-shaped appearance. Ventrally the ectodermal cells of the first and second segments present very much the appearance of this tissue in the mature stolon, but in the third segment the spindle cells (*c.e.p.*) again appear, and this tissue assumes an appearance similar to that in the zone of proliferation. The mesoderm presents dorsally and, less distinctly, ventrally a coelomic cavity for segments 1 and 2, but in segment 3 the cavity is wanting.

In these three segments, which have in Pl. XIII, Figs. 6 and 17, been designated as stolon A, the external appearances suggestive of a distinct stolon have been confirmed by the internal structures of these segments; and, these segments being typical of the very earliest stolon differentiation, there can, therefore, be recognized in their structures: (1) a thickened ectoderm from which the head will later on be differentiated; (2) an anal segment of purely embryonic tissue which, as will be seen later on, contributes to the elongation of, (3), the indifferent region in which the ordinary setigerous segment is being formed. All of these segments are, as may be seen in the study of longitudinal sections of the different stages in the development of these segments, derived from the region of proliferation, so that all the tissue of that region which I have designated as the embryonic region owes its origin to the single center of growth within the last segment of the parent stock.

#### *Development of the Head and Buccal Segment.*

The first differentiation toward the development of the head makes its appearance in the thickened dorsal ectoderm of stolon A (Pl. XIII, Fig. 17, *c.*), already referred to in the preceding description. In stolon I of the same figure this dorsal thickening (*c.*) has become more pronounced, and in its structure may be seen, for the first time, the fully developed spindle cells as they appear in the early stages of this dorsal thickening. Here the cells have become quite long, and are compactly arranged to form a somewhat rounded dorsal elevation of the ectodermal tissue.

Posterior to this region, in the second segment of the stolon, the ectodermal cells are less spindle-shaped and are arranged in a single row, while more posteriorly they present the characters of ordinary epidermal tissue. Anterior to this thickening, however, the dorsal ectoderm comprising the anal segment of stolon A, as we have seen in the study of the embryonic region, is also thickened and presents similarly arranged spindle cells. Since these cells appear in regions in which either nerve cells or simple epidermal cells will be differentiated, they cannot be regarded as being of a purely nervous character, even though they attain their greatest dimensions in positions which later on will be occupied by nerve cells. On the other hand, they may rather be regarded as the typical embryonic cells of this ectodermal tissue in general, the outlines of which are more easily distinguished in regions where the ectoderm is thickest. The appearance of the tissue beneath the ventral cord in the embryonic region would also support this view.

Pl. XIV, Fig. 22, presents the appearance of the developing head of stolon I in transverse section, in the plane of F, Pl. XIII, Fig. 17. Dorsally the ectoderm is considerably thickened and presents two lateral prominences which mark the lateral head regions, while between these is a small ectodermal projection which indicates the origin of the median tentacle (*d.m.*). Laterally the ectoderm is also somewhat thicker, though this becomes less marked toward the ventral surface. The outlines of the spindle cells, as in all other transverse sections, are quite indistinct; but in the lateral prominences, particularly near the surface, the nuclei of these cells (*c.n.*) are very much more numerous than in the region of the median tentacle and the dorsal mesoderm. These cell groups indicate the appearance of the first pair of eyes. Ventrally the ganglion cells (*c.n.*) present a grouping very similar to the groupings in the dorsal lateral prominences, and the cells present a very similar appearance at this stage. Along the dorsal borders the regular arrangement of nuclei in this section would suggest the presence of a distinct epidermis, as Malaquin has been able to distinguish in the mature heads of certain other species of *Autolytus*; but as this disappears in later developments, it would appear to be

of no significance. In a similar section of a stolon, a little older than stolon I, another group of nuclei has made its appearance in the cerebral tissues directly over the dorsal vessel, while the lateral groups have become somewhat denser.

Pl. XIV, Fig. 23, presents a longitudinal median section through the head of stolon 2. At this stage the cerebral thickening is very much greater than in the preceding stolon, the outline of the head has become quite distinct, and its division from the tissues of the anal segment is quite well marked. The median tentacle (*d.m.*) appears quite prominent, and its internal structures are not unlike the surrounding structures of the head proper. The cerebral tissues have, however, at this stage already begun to be differentiated into a small central medullary region (*c.b.*) and the peripheral cortical area (*c.n.*) of the mature head. The medullary region has appeared directly over the mesodermal tissue in the region occupied by the group of nuclei, which I have described as appearing in a line with the median tentacle in a transverse section of a stolon a little older than stolon I, and owes its origin to changes which have taken place within this group of nerve cells. Examined under a high power, this young medullary tissue presents an appearance similar to the tissue along the border of the medullary substance in the head of the mature stolon, and does not as yet possess the dense fibrillated arrangement common to the medullary substance of this region in more mature individuals. The cortical substance in this plane is composed of cells evenly distributed throughout the region of the head and showing no differences in the median tentacle. The arrangement of the nuclei for the formation of a distinct epidermis over the anterior part of the head is not as evident as it was in the transverse section of the preceding stolon; but in the median tentacle not only have the nuclei an arrangement suggestive of epidermal tissue, but the outlines of the cells are also more plainly visible, and form a marked contrast with the underlying cells. Posterior to the median tentacle the ectodermal cells still retain their embryonic spindle characters, and this region is already beginning to contribute toward the formation of the buccal segment.

The mesoderm beneath this thickened tissue has already undergone some changes. Posterior to the medullary substance and beneath the area in which the spindle cells blend with the brain cells, a new growth of mesodermal tissue (*c.me.*) has made its appearance and is pushing up toward the base of the median tentacle. This tissue results as an outgrowth from the mesoderm of this segment and will form the musculature of the dorsal median and the other tentacles of the head.

A transverse section of the head of a stolon of this age, passing through the plane G of the figure just described, is represented in Pl. XIV, Fig. 24. [This section is not exactly transverse, and ventrally includes parts of the anal segment and caudal cirrus (*c.c.*)] Dorsally the brain cells (*c.n.*) form a thick mass of tissue which projects considerably beyond the lateral margins of the segment. On either side of the plane occupied by the eyes, the anterior pair of which are quite well developed, the brain cells are uniformly distributed, and along the external border so irregularly placed as to give no indication of the presence of an epidermal formation. The medullary substance (*c.b.*) appears as a small area immediately overlying the faint dorsal muscle fibers (*m.d.*) and does not project beyond the lateral border of the mesoderm.

Pl. XIV, Fig. 25, represents a section through the posterior part of the head of stolon 2 taken in the plane H, Fig. 23. In this region the cortical substance is less prominent than in the preceding section, and the ganglion cells (*c.n.*) are less abundant. Along the dorsal margins, as well as laterally, the nuclei of these cells are again regularly arranged and seem to form a layer of tissue that is directly continuous with the epidermis over the parapodia (*p.*). The medullary substance (*c.b.*) forms a narrow plate in this region and is also less prominent than in more anterior sections. Laterally the cerebral tissue in this region gradually blends with the less thickened ectodermal tissue of the first setigerous segment, and as yet shows no signs of the circumoesophageal nerve ring.

Beneath the cerebral tissue, on either side of the area of medullary substance, a small cavity (*coe.*!) has made its appearance. This cavity is the forward extension of the coelomic



cavity (*coe.*) on the first setigerous segment, and has been formed by the upward growth of the mesoderm for the formation of the tentacular muscles, a part of which tissue (*c.me.*) may be seen on either side of the dorsal blood vessel in this section.

The next important stage in the development of the head is represented in a longitudinal median section of stolon 4 (Pl. XIV, Fig. 26). At this stage the structures of the head, as has been seen in the dorsal view of this stolon (Pl. XIII, Fig. 6), have become quite well developed, and the rounded mass of cerebral tissue presses down into the underlying tissue, so as to appear partly imbedded within it. At the same time the line of junction of the cerebral tissue and ectoderm of the anal segment anterior to it, which, as seen in Pl. XIV, Fig. 23, appeared as a deep groove, has by the increase in the size of the head been completely covered, and is now placed almost ventral to the middle region of the head. The head itself in this section appears quite distinct from the surrounding tissues, and protrudes considerably beyond the lower limits of the stolon, the tissues directly underlying it being those belonging to the region of separation. The buccal segment (*b.sg.*) has also appeared, and forms a distinct segment separated posteriorly from the first setigerous segment (Sg. 1) by a dissepiment, while anteriorly it is indistinctly separated from the structures of the head and anal segment by the mass of undifferentiated mesoderm occupying this region. The cortical substance of the head is clearly defined from the surrounding structures, and the brain cells are somewhat more densely crowded than in the preceding sections, while the arrangement of the peripheral cells suggestive of the presence of an epidermis has disappeared, and a distinct epidermis in the head structures of this stage of development is visible only in the tentacles. The medullary substance (*c.b.*) has become more prominent, and dorsally has sent up two processes which at the base of the median tentacle unite and form the nerve (*n.t.*) of the tentacle. Similar processes, as may be seen in more lateral sections of this stolon, are also sent out to innervate the lateral tentacles and the eyes.

The ectoderm on the dorsal surface of the buccal segment, as is also the case in the anterior part of segment 1, is still thickened and somewhat embryonic in character; while in the anterior ventral region the faint outline of spindle cells may be seen immediately posterior to the degenerating tissues of the region of separation (*r.s.*). A short time after the buccal segment has attained its full length, the dorsal cells give way to a distinct epidermis and ventrally to the ganglia cells common to this stage. The mesoderm of the buccal segment has been differentiated into a somatic and splanchnic layer, and both ventrally and dorsally a distinct coelomic cavity is present throughout the greater part of the segment. In the anterior region, however, the cavity in this section is broken up by the musculature of the median tentacle (*c.me.*), but in more lateral sections it is in direct communication with the cavity of the head already present in stolon 2 (Pl. XIV, Fig. 25).

The development of the circumoesophageal nerve ring in the ectodermal tissues of the buccal segment is very similar to the development of the medullary substance in the head. It first appears in a stolon of a stage of development a little earlier than stolon 4, and at this age it has already placed the brain into direct communication with the ventral cord. In a transverse section of the head of this stolon, passing through the anterior basal parts of the median tentacle and ventral tentacular cirri (Pl. XIV, Fig. 27), the more dorsal parts of the circumoesophageal nerve ring (*c.oes.*) may be seen on each side as a branch of the medullary substance extending through the mass of nerve cells directly over the mesodermal tissue; while external to these branches, just as over the medullary substance in the brain, are placed a number of nerve cells (*c.n.*), some of which will in the further development of the stolon add to the thickening of the nerve ring, while the others will remain as the nerve cells that surround these nerve fibers in the mature stolon. In the ventral ectoderm of this figure, which represents a part of the region of separation (*r.s.*), the ectodermal cells have undergone a partial degeneration, thus obscuring the ventral cord and the cells beneath it; and it is just posterior to this region that the nerve ring blends with the ventral cord.



As may be seen in the earlier stages of the development of the nerve ring, the nerve fibers composing this ring make their appearance as downward growths from the medullary substance of the brain, while the fibers of the ventral cord remain passive.

The remaining important stage in the development of the head consists in the shifting of the anterior eyes from the dorsal surface of the head to a position almost directly ventral to the posterior eyes. The change in position of the eyes first becomes apparent in a stolon a little younger than stolon 4. At this stage a transverse section in a plane with the anterior eyes will reveal a mid-dorsal area of rapid growth, extending from this plane forward to the anterior part of the head. In a transverse section of the head of a stolon, corresponding in development to that of stolon 5 (Pl. XIV, Fig. 28), this growth has already been sufficiently great to carry the anterior eyes (*e.a.*) from their original dorsal position to a lateral ventral position, and the lens (*l.*), instead of being directed upward, is directed almost directly downward. In later stages this process will be continued by the active increase of the brain cells in the mid-dorsal region (*cn.d.*) until the anterior eyes occupy a position almost ventral to the posterior eyes, the complete location of the eyes taking place immediately after the separation of the stolon.

The development of the eyes in the stolon of *Autolytus varians* is so similar to the development of the eyes in the embryo, and so identical with that described by Andrews (13), that I shall not enter into a description of this process.

#### *Development of the Mesodermal Structures.*

The development of most of the mesodermal structures of the stolon is also identical with the development of similar organs in the embryo, and hence needs but little mention here. The mesodermal tissue of the embryonic region, even in the youngest segment, is not uniformly embryonic throughout, for at this stage there may already be distinguished in the dorsal regions indistinct bands of sparsely scattered muscle fibers, which are a continuation of the dorsal musculature of

the last segment of the parent stock. Later these fibers are increased in number by additions of tissues from the dorsal dense area of cells (Pl. XIII, Fig. 18, *m.e.'*), and at the same time the tissues of this region divide into the somatic and splanchnic layers, so that at a slightly older stage (Pl. XIV, Fig. 21) there is present a dorsal coelomic cavity (*coe.*) with distinct overlying muscle tissue dorsally (*m.d.*), while laterally it gradually merges into the more differentiated tissue. The ventral dense area of the embryonic mesodermal cells, seen in Pl. XIII, Fig. 18 (*m.e.ii*), still persists in Pl. XIV, Fig. 21 (*m.e.ii*), and it is from this region of cells that the reproductive organs and nephridia will be formed. The nephridia can very frequently be distinguished in a stage of development represented by stolon 2, but more frequently they can be clearly defined only in later stages.

The male reproductive products may be detected in small numbers in a stolon corresponding in external development to stolon 4. A little later these sperm cells become quite numerous, and in a transverse section of the second setigerous segment of a stolon of the age of stolon 5 (Pl. XIV, Fig. 29, *s.p.*) they occupy a great part of the body cavity. At a later stage these cells become still more abundant and completely fill the body cavities of the first three setigerous segments, and at the time the stolon is liberated from the chain the spermatozoa have completely ripened.

The ova may be distinguished in the developing stolon even at an earlier stage than the male reproductive products, and not infrequently in a stolon in which the head is just beginning to appear small ova may already be distinguished in the lateral body cavity. Near the time when the stolon separates from the chain, the ova become so numerous as to completely fill the body and even fill the cavities of the parapodia, after which, by the relaxation of the ventral tissues, caused by the pressure of the increasing ova upon this region, the egg-sac is formed, and the ova from all parts of the body are transferred into this sac.

## ELONGATION OF THE STOLON.

After the outlines of the young stolon (A, Pl. XIII, Fig. 6), formed entirely of tissues derived from the zone of proliferation, have appeared, it has been seen in the external description of the phenomena of stolonization that a new region of growth makes its appearance; and that it is this region, which I have designated as the *region of elongation*, that contributes to the increase in the number of setigerous segments of the stolon. The external appearance of the segments of the embryonic region in its different stages of development up to a stage of differentiation equal to that of stolon A indicates, according to the description I have given, the absence of such a mode of segment formation. This fact is also supported by the appearance of the tissues of these segments in a longitudinal section (Pl. XIII, Fig. 17), where in the ventral ectoderm the embryonic cells (*e.e.p.*) in every section I have examined are confined to a single segment, which, as has been seen, forms the anal segment of the forthcoming stolon. In a longitudinal section through the last two segments of the stolon represented in Pl. XIII, Fig. 7, in which a new segment formed by this region first occurs, the ectodermal and mesodermal structures of both segments are exactly like the structures of the anal segment of stolon A, Pl. XIII, Fig. 6, the two segments being distinguishable from one another only by their external constriction, and hence composed of embryonic tissue. A little later, however, the tissue of the newly formed segment (*x*) undergoes a higher differentiation, the spindle cells disappear, a coelomic cavity appears in the dense mass of mesoderm, and the segment presents in a longitudinal section an appearance very similar to that of the segment (Sg. 1) in the embryonic region of Pl. XIII, Fig. 17. The anal segment, in the mean time, retains its embryonic characters, begins to elongate, and before long adds another segment to the series. Different stages of this process are represented in the longitudinal sections in Pl. XIII, Fig. 17, Pl. XIV, Figs. 22 and 25, and sufficiently explain the descriptions of Pl. XIII, Fig. 7, to make additional figures useless. In all these sections it will be seen that the anal segment is the only seg-

ment of the setigerous series that retains the purely embryonic characters, the segments preceding it, in very young stolons at least, as soon as formed undergoing a sufficient differentiation to make the distinction quite marked. This is particularly evident in the stolons represented in Pl. XIII, Fig. 17, and even in Pl. XIV, Fig. 23, where a new segment has just been formed; and in these stolons it may be safely said that the anal segment forms exclusively the region of elongation. In stolons 2 and 3, where elongations of the stolon by the addition of new segments are very active, the tissues of the young segments anterior to the anal have, in longitudinal section, an embryonic appearance similar to that of the anal segment, and in these segments the different tissues, as well as the external constrictions in the mid-region of some of these segments, indicate the possibility of an increase in the number of segments by division of some of the newly formed setigerous segments. Later on, however, when the formation of new segments becomes less rapid, the embryonic tissues are again confined to the anal segment (Pl. XIII, Fig. 26), and in all stages of development the tissues of the anal segment appear to be active centers from which are developed the new segments of this region. The center of growth in the anal segment is similar to that in the region of proliferation in the last segment of the parent stock, and in examining the anal segments of stolons in different stages of development it will be found that the embryonic tissues are retained as long as new segments are added to the stolon.

Malaquin (9) regards the formation of new stolons in the posterior part of the stolon as taking place in a region anterior to the anal segment which he calls the *Zoonite formateur*. In describing the formation of new segments in the stolon of Myrianida he says: "Il semble donc très probable que ce phénomène est bien constant et que la production de stolons se fait chez la Myrianide dans la zone terminale même d'accroissement de l'adulte, c'est-à-dire dans la zone précédant immédiatement le pygidium, et que j'ai désignée déjà sous le nom de *Zoonite formateur*. . . . La zone active de prolifération, le zoonite formateur, est donc bien distinct du pygidium, en tant que zoonite; si, en effet, le pygidium avait le pouvoir de former



les segments nouveaux, s'il renfermait les tissus embryonnaires en voie active d'accroissement, la zone de prolifération des nouveaux segments, et par conséquent des nouveaux bourgeons, serait irrémédiablement entraînée avec lui. D'autre part, nous verrons plus loin qu'il existe des zoonites formateurs très éloignés du pygidium et absolument indépendants de ce dernier." In describing the stolon of *Autolytus edwarsi* he makes the same observation: "Il est inutile d'insister ici sur l'accroissement du corps, c'est-à-dire sur la formation de nouveaux anneaux, qui est toujours identique à ce qu'elle est partout.

"C'est le *Zoonite formateur* qui en se multipliant, produit de nouveaux anneaux et fait s'accroître le corps d'avant en arrière. Les caractères de l'individu et de l'espèce restent identiques pendant cette *période d'accroissement* qui s'étend de l'individu jeune à l'individu adulte possédant 50 à 60 segments."

My observations in sections of *Autolytus varians* do not accord with this view. In young stolons corresponding in age to stolon 1, Pl. XIII, Fig. 6, sections of the posterior part of the stolon plainly indicate the absence of any embryonic tissue anterior to the pre-anal segment (Pl. XIV, Fig. 23), and hence the absence of a region in which the formation of new segments could take place. The pre-anal segment in this stolon furthermore presents the appearance of having quite recently been separated from the anal segment, this accounting for the embryonic tissue seen in this segment, and also indicating the manner in which new segments make their appearance. In stolons with a greater number of segments than the one just referred to, the pre-anal and several segments anterior to it do contain embryonic tissues very little more advanced in the more anterior segments than those of the anal segment itself; and it is in such segments that we found traces of external segmentation that would indicate the formation of new segments in a region anterior to the anal segment. In older stolons, however, where the formation of new segments is no longer as rapid, this embryonic tissue (Pl. XIV, Fig. 26) is again confined to the anal segment and new segments must hence be derived from this region. Since the anal segments contribute the very first segments toward the elongation of the

stolon and also take part in the formation of the last segments that are added to the almost mature stolon, the formation of new segments in a region anterior to the anal segment must be regarded as a secondary process and not the primary factor in the elongation of the stolon; and the *Zoonite formateur* cannot in this species, at least, have the significance that Malaquin attributes to it in the Syllidians in which he has made these observations.

#### SEPARATION OF THE STOLON.

As the young stolons of the chain become more differentiated, it has been seen in the lateral and ventral views of the united stolons that the region of the anal segment adjoining the head parts of the succeeding stolon becomes somewhat distinct from the anal segment in external appearance, and forms what I have designated as the region of separation. In sections this region forms in young stolons an indistinguishable part of the anal segment, and even in older adjoining stolons, in which it is externally visible as a distinct band, sections of this region merely show a decrease in the amount of mesodermal tissue ventrally and laterally (Pl. XIV, Fig. 23, *r.s.*), and a similar decrease in the entire ectodermal tissue. (The ventral projection of the ectoderm in this section is due to abnormal contractions.) The tissues of this zone, however, do not remain as an intrinsic part of the anal segment, but at a later stage are made distinct by the appearance of a degenerative process in the ectodermal cells, particularly in the ventral region (Pl. XIV, Figs. 26-28, *r.s.*). This degeneration of the ectodermal tissue in the ventral region also extends to the nerve cord, effects its severance, and at the same time so weakens the tissues in this place that, by the independent movements of the stolon, separation from the chain is readily accomplished.

Claparède (4), de St.-Joseph (7), and Pruvot (8), in different species of Syllidians have described the separation of the stolon as taking place between two segments. In chain-bearing Syllidians, like *Autolytus varians*, in the separation of its stolons from the chain, it appears to me incorrect to make this statement; for, even if the zone of separation be regarded as an











intrinsic part of the anal segment, this segment, as compared with the other segments of the stolon, is so embryonic in character, and its posterior outlines so poorly defined, that at the time of separation it has hardly attained the value of a distinct segment. The appearance of this region in longitudinal section at once seems to suggest that the separation of the stolon does not take place in the tissues forming a part of a true segment, but rather in a mass of embryonic tissue, which in the development of the head and buccal segment has been formed as an embryonic union of these and the anal segments, and constitutes a narrow undefined band of embryonic tissue anterior and posterior to which similar embryonic cells are undergoing differentiation, while at the same time the embryonic cells of this region are beginning to degenerate. The embryonic regions of the buccal and anal segments are hence continuous, and it is in the midst of this undifferentiated region that the degeneration of the cells appears and separation takes place.

#### POSITION OF THE CHAIN.

The position of the chain of stolons in *Autolytus varians*, as I have already indicated, is not constant, but varies considerably in different specimens. Like the chain-forming species, *A. ehbiensis* and *A. edwarsi*, described by de St.-Joseph, the region of stolonization in this species has a similar range, although the position of the chain is never as far forward as in the species described by this author. In a hundred specimens examined, the greater number of chains were found to be attached to segments between the 32d and 38th setigerous segments. A small number bore the chain posterior to segments 45-48, and a still smaller number bore the chain of stolons as far forward as segments 23-28. A few specimens were found, in which the chain was placed as far forward as the 19th and 21st setigerous segments, while a few others were also found, in which the chain was borne as far back as segments 56 and 58. The range of stolonization in this species, therefore, is between setigerous segments 19-58.

In describing the position of the chain of stolons, Malaquin and de St.-Joseph appear to regard this great difference in the

position of the chain in the same species as being accidental rather than due to any particular cause. The appearance of several specimens observed, and the difference in size and apparent age of the parent stock in individuals in which there is a great difference in the position of the stolon, together with the fact that in *Autolytus cornutus* and in *Procerea* the position of the stolon is so constant, has caused me to regard the range in the position of the chain as being due to more than mere accident. In this species there is, without a doubt, a distinct difference between the age of the parent stock with 56 or 59 segments and that of 19 or 20 segments. This is indicated, both by the size and more mature appearance of the parent stock, in which the largest number of segments occur, and also by the distribution of the red color-spots, which equally indicate the difference in age between the young individuals without stolons, or those in which stolonization is just appearing, and the chain-bearing individuals. Furthermore, specimens are occasionally found in which the line of demarcation between the posterior segment of the parent stock and the anterior segment of the chain is not sharply defined by the great difference in the size of the segments as it is in the specimens usually found. One specimen of this kind which I have observed showed an anterior segment of the chain that was very much larger than the second segment of the chain, and had attained a size nearer to that of the last segment of the parent stock. No parapodia were present in this segment, and the only indication of a condition differing from that of the ordinary was the increased size of the segment. In another specimen, however, I found a similarly located segment bearing parapodia, and presenting all the characteristics of a segment of the parent stock, but the small size of the parapodia, and the less mature appearance of the segment, gave evidence of its having more recently been added to the parent stock. Such specimens would accordingly indicate that the embryonic region not alone furnishes segments for the formation of stolons, but also contributes segments for the elongation of the parent stock.

Evidence that additions to the number of segments of the parent stock may occur abnormally can occasionally be obtained



from previously injured specimens. Pl. XIII, Fig. 19, represents the posterior part of such a specimen of *Autolytus varians* with a chain of stolons attached to the 44th setigerous segment, presenting the normal phenomena of stolonization. In this individual, presumably after injury, a second series of embryonic segments has made its appearance between segments 40 and 41, and consists of six well-marked segments, in every way similar in structure to that of the embryonic region of a normal chain of stolons. All the segments have advanced sufficiently far in the course of development to indicate their future condition, and there can without difficulty be marked out a developing stolon (St. 1), in which the segment from which the head will be formed has already developed well-marked parapodia. Segment 41 has also undergone changes, and gives unmistakable evidence of a head formation, thus converting the four segments of the parent stock (41-45) into part of another stolon. Anterior to stolon I is a segment (*r.e.*) which is similar to and represents the embryonic segments of an ordinary chain. Anterior to this is a segment (*s.a.*) which is larger than the embryonic segment, and in addition shows developing parapodia, thus demonstrating without a doubt that it is being added to the parent stock.

This figure gives additional evidence that the zone of embryonic segments contributes segments, not only posteriorly for the formation of the stolon, but also anteriorly for the lengthening of the parent stock. In this way I think the great length of some of the parent stocks (a length which I have not been able to find in slender specimens) may be explained, and in consequence the process of stolon formation in this species may be regarded as being confined to a range between segments 18 to 38, the range in which I have noticed the formation of the first stolon of the chain in young individuals, and not limited to accidental occurrence between segments 18 and 51.

#### ORIGIN OF THE CHAIN.

The first indication of the phenomena of stolonization appears in a young individual from 8 to 10 mm. in length and

containing from 40 to 48 setigerous segments, all of which, with the exception of the anal, are similar in structure, and many of which have reached a stage of development equal to that of the segments of a chain-bearing parent stock. It consists in a thickening of the ectoderm of one of the segments posterior to the 18th to 38th, and the subsequent development of the head in a manner very similar to the development of the head in the embryonic segments of the chain. The appearance of the head at once marks a division of the individual into an anterior parent stock, and a posterior stolon formed of segments originally belonging to the young individual and hence formed in a manner different from the formation of the stolons of the chain. The manner in which this stolon is formed has been described as that of fission (*scissiparite*, de St.-Joseph) in forms in which the production of a single stolon is followed by its separation and a subsequent regeneration of separated parts. In chain-bearing forms, however, this so-called fission, besides producing a stolon that is different from the stolons separated from the chain, does not consist of the separation of the stolon after it has matured; but in the course of the formation of the head and buccal segment, new tissues and later new segments begin to appear between the segment upon which the head has been formed and the segment anterior to it. In this way the stolon is carried back just as is the case in the maturing stolons of the chain, and finally becomes separated as the first stolon of the newly formed chain. Before the stolon has separated, the new segments may have become sufficiently numerous and their development advanced sufficiently far to show the presence of one or two distinct stolons similar in appearance to the youngest stolon represented in Pl. XIII, Fig. 6. Subsequently, while these young stolons are maturing, the chain is elongated by the addition of new segments and the development of new stolons in the manner described by de St.-Joseph and Malaquin in similar chain-bearing species as that of budding (*bourgeoisement*).

Stolonization accordingly makes its appearance in the young so-called asexual individuals in the formation of a single stolon, by a process somewhat similar to that of fission in chainless

forms. This is followed by the successive formation by budding of a number of stolons which owe their origin, as has been seen, to an outgrowth of tissue from the last segment of the parent stock. The maturing and separation of the stolon would complete the cycle of stolonization in this species, but frequently specimens may be found which give evidence of the formation of a second stolon by fission anterior to the position of the chain and at the expense of the segments of the parent stock. This stolon appears after the stolons of the chain have all been separated, the remaining embryonic segments of the preëxisting chain forming the posterior region of this stolon. The formation of such a stolon in this species is always by true fission, such as described by de St.-Joseph; and in no instance have I found an embryonic region indicating the formation of a chain as occurs in the development of the first stolon.

The cycle of stolonization in *Autolytus varians*, therefore, consists in :

1. The development of a first stolon on the young asexual individual by a process akin to that of fission.
2. The development of a chain of stolons from the last segment of the parent stock by the process of budding, and the successive separation of an unknown and possible variable number of stolons.
3. The development of possibly a single stolon posterior to the middle region of the parent stock by a true fission.

The subsequent fate of the parent stock from which the last stolon has been separated by fission and which may consist of from 19 to 24 setigerous segments, I have not been able to satisfactorily determine in this species. Some of these individuals may be found to contain eggs as far forward as the segment posterior to the gizzard, thus suggesting the conversion of the parent stock into a sexual individual by a process similar to that described as *Epigamie* by Malaquin, to which I have previously referred in another species (14). None of the specimens found, however, would warrant this assumption.

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<i>ac.</i>	Aciculus.	<i>e.a., e.p.</i>	Anterior and posterior eyes.
<i>a.l.</i>	Anterior lateral antennae.	<i>ect.</i>	Epidermis.
<i>a.s.</i>	Anal segment.	<i>e.e.</i>	Ectoderm.
<i>b.sg.</i>	Buccal segment.	<i>ent.</i>	Entoderm.
<i>c.</i>	Head.	<i>l.</i>	Lateral tentacles.
<i>c.b.</i>	Medullary substance.	<i>m.ac.</i>	Muscles of parapodia.
<i>c.c.</i>	Caudal cirri.	<i>m.d., m.v.</i>	Dorsal and ventral muscle bands.
<i>c.n., c.m.'</i>	Ganglion cells.	<i>m.e.</i>	Mesoderm.
<i>coe.</i>	Coelome.	<i>ne.</i>	Nephridia.
<i>c.oes.</i>	Nerve ring.	<i>n.t.</i>	Nerve tentacle.
<i>cu.</i>	Cuticle.	<i>o.</i>	Mouth.
<i>d.c.</i>	Dorsal cirri.	<i>p.</i>	Parapodia.
<i>dis.</i>	Dissepiment.	<i>p.l.</i>	Posterior lateral tentacles.
<i>d.m.</i>	Dorsal median tentacle.	<i>r.e.</i>	Embryonic region.
<i>d.r., v.r.</i>	Dorsal and ventral ramus of parapodium.	<i>r.s.</i>	Region of separation.
<i>d.t., v.t.</i>	Dorsal and ventral tentacular cirri.	<i>s.oeg.</i>	Suboesophageal ganglion.
<i>d.v., v.v.</i>	Dorsal and ventral blood vessels.	<i>s.p.</i>	Sperm cells.
		<i>t.m.</i>	Tentacle muscles.
		<i>v.c.</i>	Ventral cord.



## EXPLANATION OF PLATE XIII.

FIG. 1. *Autolytus varians*. Parent stock with a chain of five distinct stolons.  $\times 5$ .

FIG. 1. *a*. Enlarged parapodium of the parent stock.

FIG. 1. *b*. Enlarged setae of the parent stock.

FIG. 2. Enlarged head of the parent stock.  $\times 15$ .

FIG. 3. Mature male stolon.  $\times 30$ .

FIG. 4. Parapodium of the mature male stolon.

FIG. 5. Mature female stolon.  $\times 30$ .

FIG. 6. Chain of six distinct male stolons.  $\times 18$ .

FIG. 7. Very young isolated stolon.

FIG. 8. Lateral view of the posterior part of stolon 1 and the anterior part of stolon 2.  $\times 40$ .

FIG. 9. Ventral view of the adjacent parts of stolons 2 and 3.

FIG. 10. Parapodia of stolon 4.

FIG. 11. Lateral view of the posterior part of stolon 3 and anterior part of stolon 4.  $\times 40$ .

FIG. 12. Parapodia of stolon 5.

FIG. 13. Parapodium of a mature female stolon.

FIG. 14. Longitudinal median section through the head of a mature male stolon.  $\times 140$ .

FIG. 15. Transverse section through the last segment of the parent stock in the region of a parapodia.  $\times 200$ .

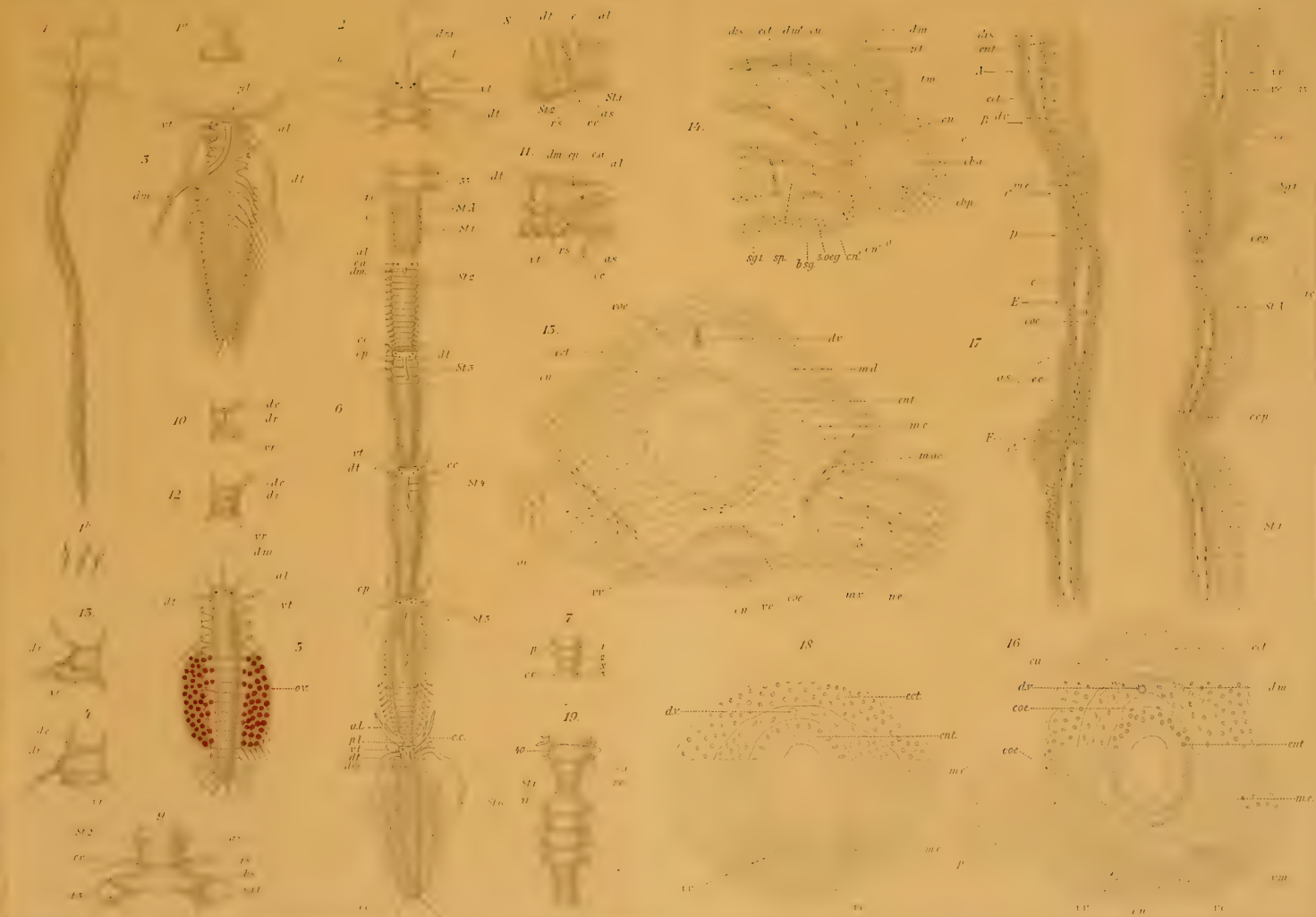
FIG. 16. Transverse section through the posterior part of the last segment of the parent stock.  $\times 200$ .

FIG. 17. Longitudinal median section through segment 35, the embryonic region, and the anterior part of the stolon 1, Fig. 6.  $\times 175$ .

FIG. 18. Transverse section through the anterior segment of the embryonic region of the chain.  $\times 200$ .

FIG. 19. Posterior part of a specimen showing the formation of new segments between segments of the parent stock.  $\times 18$ .









## EXPLANATION OF PLATE XIV.

- FIG. 20. Transverse section through the second segment of the chain.  $\times 200$ .  
FIG. 21. Transverse section through the posterior part of the first segment of stolon A.  $\times 175$ .  
FIG. 22. Transverse section through the head of stolon 1.  $\times 200$ .  
FIG. 23. Longitudinal median section through the posterior part of stolon 1 and the anterior part of stolon 2.  $\times 175$ .  
FIG. 24. Transverse section through the anterior part of the head of stolon 2.  $\times 175$ .  
FIG. 25. Transverse section through the posterior part of the head of stolon 2.  $\times 175$ .  
FIG. 26. Longitudinal median section through the anal segment of stolon 3 and the anterior part of stolon 4.  $\times 175$ .  
FIG. 27. Transverse section through the head and buccal segment of stolon 4.  $\times 200$ .  
FIG. 28. Transverse section through the anterior part of the head of stolon 5.  $\times 200$ .  
FIG. 29. Transverse section through the second setigerous segment of stolon 5.  $\times 200$ .















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## ON THE HEART OF LUNGLESS SALAMANDERS.<sup>1</sup>

HENRY L. BRUNER, PH.D.

IN the *American Naturalist* for 1896 Hopkins (14) announced the discovery of a septum atriorum in the heart of certain lungless salamanders. Beginning with the heart of salamanders with lungs, Hopkins calls attention to various parts of that organ, but omits entirely the valve at the sinus-atrium opening. Later, however, in his study of the heart of the lungless salamanders, Hopkins observed this valve, but considered it to be a rudimentary septum atriorum. The conclusion of Hopkins, that such a septum is present in lungless salamanders, has been accepted by Bethge (1), but apparently without personal investigation. A similar view occurs also in the recently published "Vertebrate Zoölogy" of Kingsley (17). On account of these facts I wish to report some observations which I had already made on the heart of lungless salamanders before the paper of Hopkins came to my notice. The investigation was undertaken at the suggestion of Professor Wiedersheim, in whose Institute in Freiburg the work was begun, and to whom I am indebted for the necessary material.

### *Historical.*

The general structure of the heart of amphibians was described by Rusconi (29), Brücke (7), and Stannius (28), about the middle of the present century.

An excellent account, published somewhat later, is that of Fritsch (11), whose work is made, to a considerable extent, the basis of the description in Bronn's "Amphibien," where also the figures of Fritsch are reproduced. In his work on the frog, Fritsch studied the structure of the ventricle and the arrange-

<sup>1</sup> A short abstract with the above title was published in the *Proceedings of the Indiana Academy of Science* (3) for 1897. Later a similar notice appeared in the *Anatomischer Anzeiger* (4).

ment of valves in the heart, and pointed out for the first time the function of the spiral valve in regulating the direction of the flow of blood.

Langerhans (21) described the perforations in the septum atriorum of Urodela. His results were later confirmed by Huxley (15) and Boas (6).

Boas (5) investigated the spiral fold of the salamanders, and showed it to be formed by the fusion of a row of valves. Indications of this origin are still to be found in some forms (*Triton cristatus*), in which the valve is represented by a row of knots on the conus wall.

Gompertz (12) published an excellent account of the physiology of the anuran heart in 1884.

Langer (19) worked out the development of the proximal and distal valves of the conus arteriosus. Thickenings representing these valves appear in a very early stage of the larval development. Later these thickenings are hollowed out next to the conus wall.

The literature of the lungless salamanders begins in 1894, when Wilder (35) announced the discovery of a number of species. The same year Camerano (9) discovered two lungless Italian forms. In 1896 Wilder (36) and Lönnberg (22) each added a number of species to the list, which, according to Wilder, includes at least half of the known species of Salamandrinae.

Camerano (9) attempted to determine the mode of respiration of *Salamandrina perspicillata* and *Spelerpes fuscus*, following the method of Marcacci (37) with the frog, by hindering the bucco-pharyngeal respiration. As the animals experimented with quickly died, in spite of the fact that the cutaneous respiration was undisturbed, Camerano concluded that the latter is relatively unimportant in sustaining life, while the bucco-pharyngeal respiration is really essential thereto.

Bruner (2) found special muscles for opening and closing the external nares in the lungless salamanders. In salamanders with lungs these muscles constitute an important part of the mechanism of inspiration. In lungless forms, however, their function seems to be restricted to the protection of the



nasal cavities. The use of the muscles for respiratory purposes, such as the inflation of the pharynx or oesophagus, has, at any rate, not been observed. Study of *Salamandrina* in water also failed to show such an inflation for hydrostatic purposes.

Maurer (23) reported the occurrence of blood capillaries in the buccal epithelium of both *Anura* and *Salamandrinae* (*Rana*, *Bufo*, *Hyla*, *Salamandra*, *Triton*). Maurer is of the opinion that this vascularization is primarily a response to the demand of a many-layered epithelium for proper nourishment. The closer proximity of the capillaries to the surrounding medium would, however, also favor the more rapid aëration of the blood, and thus assist in respiration. This fact might then account for the further development of epithelial vessels for respiratory purposes (*e.g.*, in lungless salamanders).

Such a special development of the capillaries of mouth and oesophagus Bethge (1) found in the lungless *Spelerpes fuscus*. He says : "Schon mit guter Lupe erkennt man, dass sie (the capillaries) nicht glatte Gefässe sind, sondern in ihrer ganzen Ausdehnung ein fast traubenförmiges Aussehen zeigen, an manchen Stellen so deutlich, dass man einen gemeinsamen Stiel und daran sitzende Beeren unterscheiden kann. . . . Auf Schnitten durch den ganzen Kopf lässt sich die Lage der Kapillaren erkennen. Wir finden ein mehrschichtiges Epithel; die Zellen der unteren und mittleren Lage zeigen unregelmässig kubische Form, die Zellen der äussersten Schicht sind von cylinderförmiger Gestalt. Zwischen den Epithelzellen der mittleren und oberen Lage erstrecken sich Becherzellen. Die Kapillaren breiten sich nun zwischen den Zellen der basalen Lage aus und treiben Ausstülpungen zwischen die mittleren Zelllagen hinein, die häufig bis an die oberste Schicht heranreichen." This peculiar development of the capillaries must greatly facilitate respiration; nevertheless Bethge considers the respiratory function of the skin of still greater importance, and opposes the view advocated by Camerano.

Bethge compared also the larger blood vessels of *Spelerpes* with those of salamanders with lungs, and found the chief difference in the development of the pulmonary vessels. In the

lungless form the pulmonary vein is wholly wanting; the pulmonary artery, on the other hand, has been preserved because it supplies other parts than the lungs. In regard to the heart, Bethge accepts the conclusions of Hopkins.

Miss Woldt (33) found a pulmonary artery in *Plethodon erythronotus* and *P. cinereus*, in which it supplies both oesophagus and skin. Beyond the point of origin of the last oesophageal branch no trace of the true pulmonary trunk could be found.

W. E. Ritter and Loye Miller (26) find in the toes of *Autodax lugubris* great blood sinuses, which they consider an important seat of respiration. "The toes have, in fact, assumed the function of external gills."

### *The Heart of Salamanders with Lungs.*

The heart from which Fig. 1, Pl. XV, was drawn had been injected immediately after death with 70% alcohol, until all blood was washed out. The large vessels were then ligatured and the entire animal, with the heart, placed in 70% alcohol. After hardening, the heart was in excellent condition for study. Figs. 3 and 4, Pl. XV, represent sections of a heart injected with blood only. The amount of distention is less here than in Fig. 1.

The heart of *Salamandra maculosa*, which will be used as a type in the following description, is composed of two different sections—one for the reception and one for the expulsion of the blood. The former includes the sinus venosus and two incompletely separated auricles; the latter includes the single ventricle and the truncus arteriosus. The right auricle receives blood from the general circulation; the blood from the lungs pours into the left auricle. Into the latter the pulmonary vein enters directly, while the blood from the body passes first into the sinus venosus, which lies on the dorsal side of the heart and toward the left side. The sinus is formed by the fusion of three large veins—the two *venae cavae superiores*, which open into the sinus by separate mouths, and the *vena cava inferior*, which enters the sinus at its posterior end. The

sinus venosus is provided with muscular walls, whose contraction marks the first stage of the heart-beat. The return of the blood from the auricle into the sinus is prevented by a valvular contrivance, which, according to Fritsch (11), corresponds to the valvula Eustachii of higher vertebrates.

The two auricles of *Salamandra* show externally no evidence of separation. A furrow on the ventral auricular surface indicates the position of the truncus arteriosus and does not correspond to that of the septum, which lies farther to the left. An examination of the interior of the heart shows that the septum is somewhat oblique to a median vertical plane, the inclination being from left above to right below. Dorsally it attaches close to the edge of the sinus-atrium opening; caudally it hangs with a free margin over the atrio-ventricular opening. According to Langerhans, the septum atriorum of *Salamandra maculosa* is always perforated, particularly in its dorsal third. It is supplied with an abundance of muscular tissue, which contracts with the walls of the auricles.

Of the three openings in the auricular walls, two are provided with valves, the third is valveless. Of the former, the atrio-ventricular opening is guarded by two fibrous pouches, whose margins are connected by means of cords to the wall of the ventricle. The anterior surface of each valve is attached at its middle to the margin of the septum atriorum. Between the two points of attachment the septum hangs free.

The sinus-atrium opening lies in the dorsal wall of the right auricle. The plane of the opening is almost transverse to the axis of the body, but its left margin lies usually somewhat anterior to the right. Immediately in front of the opening we find the pulmonary vein, which is formed on the dorsal side of the heart by the union of two vessels, one from each lung. At the dorsal margin of the sinus opening the vein penetrates the atrial wall, on whose inner surface the now flattened vessel extends forward and toward the left, until, at the anterior margin of the sinus-atrium valve, it reaches the septum, through which it discharges into the left auricle. From the sinus opening to the septum, the vein is closely united to the atrial wall; the latter, however, is dorsal to the vein, as is shown by the struc-

ture of the entire region. This fact explains the manner in which the vein projects into the atrial cavity (see Pl. XV, Fig. 3).

The sinus-atrium opening of amphibians is furnished, in typical cases (*Perennibranchiata*), with two transversely placed valves, which are attached on the right to the atrial wall, on the left to the septum atriorum. In *Salamandrinae*, however, one of these valves, the ventral one, becomes rudimentary or wholly disappears, while the other is greatly enlarged. The form and relations of the latter valve in *Salamandra* are shown in Pl. XV, Figs. 1, 3, and 4. In this species a second valve is wholly wanting. The enlarged valve is a sail-shaped membrane, which extends from the sinus-atrium opening to the atrio-ventricular opening, where it is attached, along with the septum atriorum, to the middle of the inferior valve. The convex margin of the valve is fixed by means of muscular trabeculae to the septum; the concave margin is wholly free. The attachment of the dorsal end of the valve follows the ventral wall of the pulmonary vein from the septum to the sinus opening, where the valve bends toward the right, covers the opening, and attaches to its dorsal half.

The sinus valve, like the septum and atrial walls, is contractile, its muscular bundles being in part continuous with those of the supporting structures. In the neighborhood of the sinus opening these bundles are strongly developed in a dorso-ventral direction, which is here the direction of greatest tension.

The truncus arteriosus projects forward from the ventricle with a slightly sigmoid curve. Its proximal portion (*conus arteriosus*) contains two circles of valves—a posterior one, which lies at the ventricular opening and consists of three pouches, and an anterior set, which includes normally four valves. One of the valves of the distal circle is produced caudalward as a low ridge of connective tissue, which terminates in front of the proximal set of valves. This ridge is the so-called spiral fold of the salamander.

Beyond the *conus* we find the *bulbus arteriosus*, from which arise the great arterial trunks.

With the foregoing description as a basis for comparison, we may now turn to the study of salamanders without lungs.



*The Heart of Lungless Salamanders.*

In the following description I shall use *Salamandrina perspicillata* and *Plethodon erythronotus* as types; other forms, however (*Plethodon cinereus*, *Desmognathus fusca*, *Spelerpes fuscus*), have essentially the same structure. The preparation followed in Fig. 2, Pl. XV, was treated as that used for Fig. 1. The sections represented in Figs. 6 and 7, Pl. XV, are from a heart naturally injected with blood.

In all of the lungless species mentioned above, the external arrangement of the parts of the heart is very similar to that of the heart of *Salamandra maculosa*; but the pulmonary vein is not to be seen, and a careful microscopic examination has failed to reveal a trace of it. Similar results have been obtained by Hopkins (14) and Bethge (1) with certain lungless forms.

In the interior of the heart of *Salamandrina* still other modifications of structure may be readily seen, the most important of which is due to the disappearance of the septum atriorum, which, like the pulmonary vein, seems to have left no trace of its former existence. We find a well-developed sinus-atrium valve, which extends from the sinus opening to the ventral aspect of the ostium atrio-ventriculare; its convex antero-ventral margin, however, is fixed to the left atrial wall. The posterior end of the valve is attached to the middle of the lower atrio-ventricular valve, while its dorsal end extends from the left atrial wall obliquely caudalward toward the sinus opening, to whose dorsal margin it is fixed. This relation of the valve to the sinus opening is essentially the same that we saw in *Salamandra*, and has occasioned the remark of Hopkins, that the sinus opening of the lungless salamander leads into the left auricle.

The conus of *Salamandrina* shows the same general structure that we found in the conus of *Salamandra*. A spiral valve is distinctly recognizable in the lungless form.

Let us now consider the significance of the facts presented above. We have found in the heart of lungless salamanders certain changes of structure which must be attributed directly or indirectly to the loss of lungs. One of these changes —

the shifting of the attachment of the sinus-atrium valve — is to be accounted for by the disappearance of the septum atriorum. The valve itself shows no sign of degeneration, which indeed could not be expected to occur here, for the condition which requires the presence of a valve at the sinus-atrium opening is certainly not affected by the loss of lungs.

It is worthy of note that the loss of lungs has affected the pulmonary artery and the pulmonary vein in an altogether different way. As already stated, the survival of the artery in lungless salamanders has been reported by Hopkins (14), Bethge (1), and Miss Woldt (33). I have observed the artery also in *Salamandrina*, where, however, it receives its blood only through the ductus Botalli, the proximal portion of the vessel having disappeared. In all of the forms studied, the pulmonary artery supplies certain parts (oesophagus, skin), to which it is distributed also in salamanders with lungs. Such an accessory saving function the pulmonary vein of lungless salamanders did not possess.

The fact that the septum atriorum disappears with the lungs indicates clearly that in salamanders with lungs the septum performs a function which becomes superfluous or impossible after the loss of those organs. This function is the separation of the venous blood of the right auricle from the aërated blood of the left auricle. But what is the significance of this separation if the two sorts of blood are afterward mixed during their passage through the ventricle and conus? Or is there, after all, in salamanders with lungs, a partial separation of aërated and venous blood in its entire course through the heart? Such a separation occurs, as is well known, in the heart of *Rana*. Now as regards the atrium and ventricle we find essentially the same structure in *Salamandra* as in *Rana*. It is true that the septum atriorum of the salamander is perforated, while that of the frog is not. But during the brief stay of the blood in the auricles the small perforations which have been described in *Salamandra* would permit little mixing of the blood. There would be a much better opportunity for this to occur in the ventricle; but here we have the same spongy condition in *Salamandra* and *Rana*. So far, then, *Rana* does not seem to have



a decided advantage over the salamander in respect to the separation of venous and arterial blood in the heart. We may, therefore, conclude that in the salamander, as in *Rana*, the first blood passing from the ventricle into the conus during the ventricular systole is chiefly venous. In *Rana* this blood is directed into the pulmonary artery. In the salamander, however, the structure of the conus does not indicate that it could influence the direction of the blood current. We must turn, then, to the bulbus arteriosus and the great arterial vessels for further light on our problem. Here, however, peculiar difficulties present themselves whose solution I shall not attempt at this time. But it seems not improbable that, in salamanders with lungs, a difference of blood pressure in the arterial trunks at the time of the ventricular systole may lead to a distribution of blood similar to that occurring in *Rana*.

The spiral valve of the salamanders can have no control over the direction of the blood which passes through the conus. Its function seems to be rather to prevent the collapse and obstruction of the conus, which might, in the absence of the valve, arise either as a result of the strong contraction of the conus walls, or from outside pressure.

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## DESCRIPTION OF FIGURES.

<i>A.</i>	Undivided atrium of lungless salamander.
<i>A.A.V.</i>	Atrio-ventricular aperture.
<i>A.D.</i>	Right auricle.
<i>A.S.</i>	Left auricle.
<i>S.</i>	Septum atriorum.
<i>S.V.</i>	Sinus venosus.
<i>T.A.</i>	Truncus arteriosus.
<i>V.C.D.</i>	Right vena cava.
<i>V.C.I.</i>	Vena cava inferior.
<i>V.C.S.</i>	Left vena cava.
<i>V.P.</i>	Pulmonary vein.
<i>V.S.A.</i>	Sinus-atrium valve.
<i>P.S.</i>	Spiral fold.



## EXPLANATION OF PLATE XV.

FIG. 1. Heart of *Salamandra maculosa*, viewed from the right side. The perforations of the septum atriorum are not indicated.

FIG. 2. Heart of *Salamandrina perspicillata*, also viewed from the right side. Septum atriorum and pulmonary vein are wanting.

FIG. 3. Frontal section through the heart of *Salamandra maculosa*. The plane of the section passes through the anterior part of the sinus-atrium valve. The spongy structure of the ventricle is not shown in the drawing.

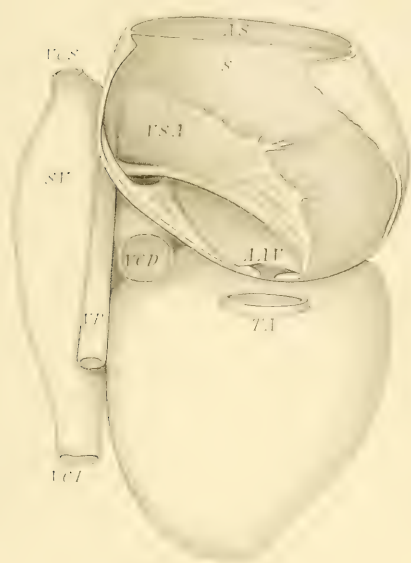
FIG. 4. Section a short distance behind Fig. 3, and from the same series. The transverse portion of the sinus-atrium valve and the dorsal margin of the sinus opening lie in the plane of the section.

FIG. 5. Section through the conus arteriosus and spiral valve of *Salamandra maculosa*.

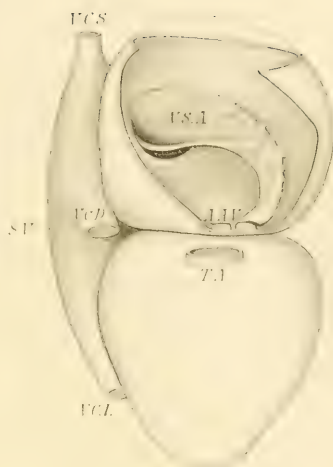
FIG. 6. Frontal section through the heart of *Plethodon erythronotus*; the direction and location as in Fig. 3.

FIG. 7. Frontal section through heart of *Plethodon erythronotus*; corresponding to Fig. 4.

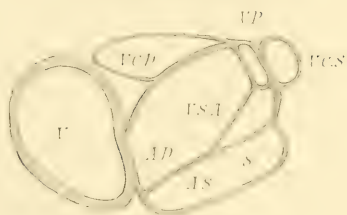
FIG. 8. Conus and spiral valve of *Spelerpes fuscus*.



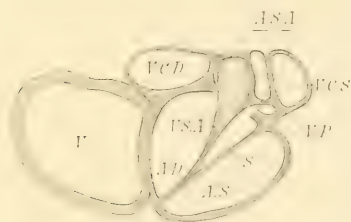
1. *Salamandra*



2. *Salamandrina*



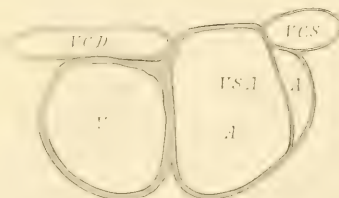
3. *Salamandra*



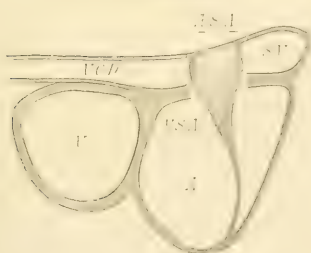
4. *Salamandra*



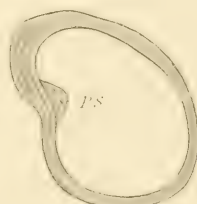
5. *Salamandra*



6. *Plethodon*



7. *Plethodon*



8. *Spelerpes*









ON THE STRUCTURE OF TWO FISH TAPEWORMS  
FROM THE GENUS PROTEOCEPHALUS  
WEINLAND 1858.<sup>1</sup>

HARRIS M. BENEDICT.

THIS paper consists of an anatomical and histological study of a little-known native species of *Proteocephalus* and an investigation of *P. filicollis*, a well-known species first described by Rudolphi, in which some additional points are elucidated.

The investigations were carried on in the years 1895-97, and the paper has been accepted as the thesis for the Master's degree at the University of Nebraska.

To Dr. Ward my sincerest thanks are due, not only for the material with which I worked and the use of his private library, but also for the valuable assistance which in other ways he has given me. I am also indebted to the kindness of Prof. Dr. Zschokke, of Basel, in sending to Dr. Ward, specimens of *P. filicollis*.

In the study of the genus of fish taeniae, to which Lönnerberg ('94) has given the name *Ichthyotaenia*, the paper by Weinland ('58) has evidently been overlooked. In this paper, which was brought to my notice by Dr. Charles W. Stiles in private correspondence, the following occurs:

"Gen. 2. *Proteocephalus*, Weinl. (The name is derived from 'πρῶτευσ,' the ever-changing principle in the old Greek mythology, and 'κεφαλή,' head.) The shape of the head of this genus is extremely changeable. There is no proboscis nor hooklets. The eggs are provided with two shells, the outer shell being mucilaginous. These taeniods live in reptiles and fishes. The type of this genus is *Taenia ambigua* Dujardin. Here belong *Taenia filicollis* and *Taenia dispar*."

. <sup>1</sup> Studies from the Zoölogical Laboratory, The University of Nebraska, under the direction of Henry B. Ward, No. 33.

I have not been able to obtain specimens of *ambigua*, and the literature which contains a more detailed description than that of Dujardin is inaccessible. Hence I could not determine from personal investigation whether *ambigua* was a true type of the genus which has been called *Ichthyotaenia*. Riggenbach ('96), however, lists *ambigua* as one of the *Ichthyotaeniae*, and the excellence of his work on this group warrants me in accepting this designation. The rules of priority in nomenclature, therefore, demand the adoption of the generic name *Proteocephalus*.

### *The Genus Proteocephalus.*

The oldest literature on the *Proteocephalus* is found in the works of Rudolphi ('10), Dujardin ('45), Diesing ('50), van Beneden ('61), and von Linstow ('78). All of these authors confine their descriptions to a few details concerning the size and form of parts, particularly of the scolex and certain proglottids.

Zschokke ('84) was the first to make any study whatever of anatomical structure, and therefore his work is of much greater value than that of the older authors. The next author to make an anatomical and histological study was von Linstow ('91), who described *T. longicollis*. He was the first to note the relationship between the various fish taeniae. In the following year Kraemer ('92) made a very exhaustive study of *Cyathocephalus truncatus*, *P. torulosa*, and *P. filicollis*, and established the identity of *P. filicollis* and *P. ocellata*. He also pointed out other common characteristics of the fish taeniae. Lönnberg ('94) was the first to establish the group under the name *Ichthyotaenia*, and made its characteristics still more distinct and accurate.

By far the best work yet done on this group is found in the paper by Riggenbach ('96), who, in addition to a careful description of two new species, has for the first time made an accurate and discriminating summation of the present knowledge of this group. At the present time a great amount of confusion exists and the accuracy of the specific determinations is doubtful in over half of the species of *Ichthyotaenia* so far described. Riggenbach shows that out of a list of twenty-nine species

only seven have supposedly been accurately determined, and this paper makes evident grave mistakes in the description of one of these forms. As an example of inaccuracy should be cited the reference by both Kraemer ('92) and von Linstow ('78) to a description of *T. torulosa* in a work of van Beneden ('61). As a matter of fact, the form described is not even given by the author as *torulosa*, but as *porulosa*, and from the figures could not possibly be *torulosa*. Until all doubtful species are further investigated, no satisfactory synopsis of the entire genus can be made.

*Proteocephalus ambloplitis* (Leidy).

The specimens of this cestode which came into my possession proved to be different in internal morphology from any carefully described species of *Proteocephalus*, and were at first believed to represent a new species. In reviewing the literature on fish cestodes, I noticed an account by Leidy ('87) of the discovery of a cestode in a rock bass, *Ambloplitis rupestris*, which he named *Taenia ambloplitis*. His description was limited to a few external measurements which did not agree with the dimensions of my specimens. He also stated, however, that the cestode closely resembled *Taenia ocellata* Rud. Dr. Ward, through the kindness of Dr. Hassell, acting curator of the Helminthological Collection of the United States National Museum, obtained for me Leidy's type specimens. An accurate study of one of these by the section method showed that in spite of certain external differences this species was identical with my specimens. This is simply another illustration of the worthlessness of a description dealing merely with external features and external measurements in the determination of the species of cestodes. Exclusive of external form and measurements, the only difference shown by a careful anatomical and histological comparison of Leidy's specimens with my own was the presence of a greater number of calcareous bodies in the parenchyma and the smaller size of the sphincter muscle. This latter particular was not important because the younger anterior proglottids in my specimens showed the same condition. This

difference then, as well as the size, indicates simply that Leidy's specimens were younger than the ones I studied. The description of this form is therefore to be emended as follows:

*Specific Characteristics.*—Total length from 280 to 410 mm. Greatest breadth from 1.75 to 2.18 mm. Surface of body very rough, with transverse ridges and furrows. Scolex prominent .82 to .88 mm. in greatest diameter just behind the posterior margin of the suckers. A minute depression, with a slight elevation in the center, is situated on the rounded apex of the scolex. The suckers are large, directed outward and forward, and separated by deep longitudinal furrows, which continue throughout the length of the short neck. Proglottids closely joined together, with edges very slightly rounded. Immature proglottids much wider than long; majority of proglottids nearly square; posterior proglottids slightly longer than wide; last proglottid with a concave posterior margin. Genital sinus irregularly alternate, situated on the margin, one-fourth of the length of the proglottis from the anterior end. Vagina with exceedingly large sphincter muscle and forming a mass of coils immediately anterior to the ovary. Cirrus formed by an invagination of the distal end of the cirrus pouch and lined by cuticula continuous with that covering the body. Vas deferens intricately coiled within the pouch and forming a very large complex mass of coils near the center of the proglottis. Excretory canals form in the last proglottis a network, which is connected with the exterior by a multitude of minute ducts passing through the cuticula.

#### *General External Morphology.*

The specimens studied were collected by Dr. Ward, while engaged in the biological examination of Lake St. Clair, during August of 1893. During this investigation (Ward, '94) ninety fish of twenty species, caught at New Baltimore on Lake St. Clair, were examined. Half of them proved to be free from cestodes, while the remaining contained two hundred and twenty-seven specimens of several species. All of the six individuals of *Micropterus dolomieu* examined were found to be infested by



this species of cestode, which was found in no other fish. The parasites occurred in the pyloric caeca and in the intestine, and varied in number from one to four in each fish. The specimens were killed with corrosive-sublimate solution and preserved in 83% alcohol—a process which gave very satisfactory results.

The specimens thus preserved varied in length from 280 to 410 mm., and the breadth of the same from 1.75 to 2.18 mm. The shape of the proglottids at the same stage of development was not the same in different individuals. Thus, in one specimen certain proglottids were nearly four times as wide as long, while in another individual those of the same development were nearly square. An individual which best represented the greater number of my specimens gave the following measurements :

Total length of body, 280 mm., containing 332 proglottids. Breadth of scolex, .84 mm.; length, .55 mm.; suckers, .4 mm. in antero-posterior diameter, .32 mm. in transverse diameter. Breadth of neck, .45 mm.

Proglottids	10 mm. from scolex			BREADTH.	LENGTH.
				.75 mm.	.08 mm.
"	25	"	"	1.2	.3
"	50	"	"	1.25	.75 to .90
"	75	"	"	1.45	.9 to 1
"	100	"	"	1.5	1.2 to 1.4
"	150	"	"	2	1.5 to 2
"	280	"	"	2	2

It will be seen from these figures that all the proglottids in the posterior half of the body are nearly of the same size; and yet in a few specimens there was a gradual and very constant increase in breadth and length from anterior to posterior ends. However, too much importance has been attached to simple external measurements by older observers, who, in many cases, limited their descriptions to statements of the size of body and the shape of scolex and proglottids. The only external measurements which are of real value are the dimensions of the scolex and the size of the unsegmented neck.

The scolex of *P. ambloplitis* is very prominent and plainly visible to the naked eye. It is .84 mm. in lateral diameter,

.80 mm. in dorso-ventral, and .85 mm. in length (Pl. XVI, Figs. 1 and 2). From the anterior face it presents a nearly square outline, with a deep notch in the middle of each side, dividing the surface into quarters. Each quarter contains a large sucker which is directed outward and upward. The apex of the head is a smooth, rounded prominence with a small depression in the top (Pl. XVI, Fig. 3). No hooks are present. A fairly good idea of the shape of the scolex can be obtained by placing two truncated pyramids base to base. One of the smaller bases will represent the beginning of the neck, the other the prominence, while on the slope just beneath this would lie the suckers. The notches seen in the anterior view of the scolex form furrows down the four sides of the head, which gradually decrease in depth and vanish on the first few proglottids.

The surface of the proglottids is very rough, being thrown into longitudinal and transverse folds, as shown in the margin of Pl. XVI, Fig. 4, where the transverse furrows are so deep as to obscure the divisions between the proglottids. The proglottids are also closely joined together, so that it is difficult to distinguish the divisions between them. The color of the body in the alcoholic specimen is a watery white, with a yellowish tinge toward the posterior end.

A small genital sinus, having a depth of .025 mm. and a diameter of .05 mm., is situated about one-fourth the length of the proglottid from the anterior end, on the margin. It is irregularly alternate in position (Pl. XVI, Fig. 4).

#### *Anatomical and Histological Structure.*

*Cuticula.*—The cuticula can be resolved into two layers; an outer thinner portion, .0015 mm. thick, forms the external surface (Pl. XVI, Fig. 11, *ct'*). It is very rough, with minute projections extending from the surface, which are coarser, blunter, and more irregular than cilia, and which stain darkly. Within this layer is another, .0075 mm. thick, which appears nearly homogeneous (Pl. XVI, Fig. 11, *ct*). This does not take the stain so vigorously as the first. Within the cuticula is a layer of transverse muscles, .001 mm. thick, which appears

as a dark line in transverse sections (Pl. XVI, Fig. 11, *m.c.ct.*). Beneath this layer is a row of dots, which mark the sections of longitudinal muscle fibers (Pl. XVI, Fig. 11, *m.l.ct.*). In frontal sections, cut somewhat obliquely, these subcuticular muscles can be best observed. Here the transverse fibers are seen to be thick and in the form of a fibrous mat, while the longitudinal fibers are separate and cross the others at right angles.

The subcuticular cells are slightly pyriform, with a large nucleus (Pl. XVI, Fig. 11, *sb.c.*). None of them are closer to the muscle layers than .0075 mm., but each sends one or more processes into the subcuticular muscle layers, beyond which they can no longer be traced.

The parenchyma cells are irregular in shape and rather closely packed, presenting the appearance of a fine meshwork with nuclei in the spaces. Scattered throughout the parenchyma are numerous calcareous bodies of various oval shapes, none larger than .007 mm. in diameter (Pl. XVI, Fig. 16, *ca.*).

*Musculature.* — The longitudinal body muscles are very prominent; they are arranged in irregular bundles, from fifty to sixty in all, which vary in thickness from .05 mm. to a fourth that size (Pl. XVI, Fig. 16, *l.m.*).

As the scolex is approached, the bundles become smaller and the fibers are not so closely compacted, many of them leaving the bundles and extending separately through the parenchyma. In the scolex the fibers are no longer arranged in bundles, but penetrate the parenchyma as scattered fibers.

Within these bundles is another muscle layer. In transverse section that portion of the proglottis which is filled with sexual organs is seen to be bounded by a thin sheet of wavy fibers (Pl. XVI, Fig. 16, *m.c.*). From the sheet single fibers dip down irregularly and pass between the organs. The ends of the fibers are coiled like a corkscrew. Other fibers go outward between the large muscle bundles and take a wavy irregular course toward the subcuticular cells. In the scolex these fibers show a radiate arrangement (Pl. XVI, Fig. 3, *m.c.*), and transverse sections in this region show the diagonal fibers crossing at the center.

The musculature of the suckers consists of three layers. Beneath the cuticular lining of the suckers is a layer of circular muscles, precisely as in the proglottids (Pl. XVI, Fig. 3, *m.s.*). Within these is the principal layer composed of fibers running perpendicularly to the surface for a distance of .065 mm. at the center and gradually decreasing to .04 mm. at the edges. Prominent nuclei are situated in the median zone of this layer (Pl. XVI, Fig. 3, *mp.*). The proximal ends of these fibers are woven in with a second circular layer of considerable thickness, but of loosely arranged fibers. A thin, dense, basement membrane covers the inner surface of the sucker.

In the body the partitions between the proglottids are sheets of muscle fibers, running transversely, with a much smaller number of dorso-ventral strands.

*Nervous System.*—In the scolex a thick band of nervous tissue is arranged in a circle, somewhat anterior to the center of the head. From a point on the ring directly beneath the lateral furrow project two branches, one to each adjacent sucker, so that each sucker receives two trunks, which enter the lining membrane at two distinct points (Pl. XVI, Fig. 8, *n.r.*). The band varies from .02 mm. in breadth, and .01 mm. thick in one specimen, to a thick mass surrounding the peculiar spherical bodies described hereafter, and having a thickness nearly twice as great as in the first instance.

From the scolex two longitudinal trunks run down the lateral margins of the proglottids just within the muscle bundles (Pl. XVI, Fig. 16, *n.*). In many transverse sections it appears as if the nerve sent off at certain places many branches of various sizes; but until live specimens can be obtained this cannot be proved. In the end of the last proglottis the nerve trunks bend toward each other and break into fine spreading fibers.

*Excretory System.*—Just behind the suckers in the scolex is a complicated system of rather regularly curving tubes of varying size and connected by curving cross branches. From the outer parts of the ducts smaller branches lead to the exterior and open by excretory pores through the cuticula (Pl. XVI, Fig. 11, *p.ex.*). From the scolex eight longitudinal excretory



ducts can be traced through those proglottids in which no sexual organs are yet developed. One pair lies in the median frontal plane, two pairs are situated below and outwards, — the last being close to the cuticula, — and one pair in the dorsal half of the body, about halfway to the cuticula. Many minute branches are given off from these which open to the outside of the body by pores .005 mm. in diameter, exactly the same as the excretory pores of the scolex. The pores which connect the excretory system with the exterior are simply minute canals through the cuticula, with no special walls. They do not resemble the very prominent pores found by Riegenbach ('96) in *P. fossata* and *Corallobothrium lobosum*, being much smaller and without any muscle fibers. They differ also in being much more numerous and entirely irregular in position.

Throughout the chain of proglottids the minute excretory tubes are connected by these pores in the cuticula with the exterior at various places in each proglottis, being especially numerous near the posterior end of each.

In the region of sexually immature proglottids a regular arrangement of the longitudinal ducts cannot be so plainly traced. The two which lie in the median frontal plane are the smallest and most regular of all. The others are larger, with many branchings and connections. A transverse section through sexually mature proglottids and those posterior shows a varying number of the sections of the ducts. From larger longitudinal tubes small branches may be traced which curve around the muscle bundles, and either terminate in branches too small to be traced or join some other branch.

Around the cirrus pouch and vaginal opening near the dorsal surface is a network of rather large ducts arising from longitudinal tubes (Pl. XVI, Fig. 15).

There is no connecting vessel at the posterior end of each proglottis, connection, as already stated, being accomplished at various places by irregular branches. This condition resembles the description by Kraemer ('92) of the excretory vessels of *Cyathocephalus truncatus*.

The cuticula of the last few proglottids is fairly honeycombed with excretory pores; especially is this true of the posterior

concave margin of the last proglottis, which resembles a sieve. The longitudinal vessels in this proglottis terminate in small sinuses, which branch and anastomose, much like the ducts in the scolex, but more irregularly. This network of ducts is in communication with the exterior by means of a multitude of pores which are found at this place (Pl. XVI, Fig. 13).

Such a posterior outlet differs from any previously described, since there is no terminal muscular reservoir. The tendency of the *Proteocephali* to the formation of excretory complexes is very well illustrated by this form.

Directly beneath the apex of the scolex is a sac of cuticular structure enclosing a small number of circular masses, closely pressed together (Pl. XVI, Fig. 3, *ca.*). The masses seem to be of a calcareous nature, and are penetrated by numerous fine canals. No connection whatever could be traced between this sac and any outside system, although the excretory ducts form a thick network about it. I have found no mention elsewhere of such a structure, which is different in many respects from the simple calcareous bodies common among cestodes.

*Sexual Organs.*—The genital sinus, as before stated, is irregularly alternate in position, and is situated on the margin about one-fourth of the length of the proglottis from the anterior end. It resembles in position and relative size that of *P. fossata*, *P. abscissa*, and *Corallobothrium lobosum* (Riggenbach, '96). In *P. torulosa* and *P. filicollis*, as described by Kraemer ('92), no common genital sinus is present, and the genital openings are exactly in the middle of the margin. The vagina opens directly in front of the male opening, a characteristic of the *Proteocephali*.

*The Male Genital Organs.*—The testes are from seventy-five to one hundred in number, arranged irregularly throughout the space within the longitudinal muscles of the proglottis (Pl. XVI, Fig. 9, *t.*). Such a position and number is described as obtaining in *P. torulosa*, *P. filicollis*, *P. abscissa*, *P. fossata*, *Corallobothrium lobosum*, and many others which have been less accurately described. When the uterus becomes distended the testes are crowded to the ventral surface, and the uterus lies in a broad irregular sheet above them. The testes are



crowded at all times enough to give each a polygonal shape. They vary in size from .05 to .065 mm. Each is surrounded by a very delicate structureless membrane, which is continuous with the wall of the vas deferens and vasa efferentia. The testes are the first organs to mature and the first to degenerate. The spermatozoa are collected by very minute ducts, which take a general direction towards the center of the proglottis, joining with others in their course. The larger ducts, which finally meet, are about .01 mm. in diameter, and these, after making more or less intricate coils, unite to form the distended vas deferens, which is .05 mm. in diameter (Pl. XVI, Fig. 9, *v.d.*). This is also coiled and twisted, forming a mass a little anterior to the center of the proglottis, extending to the cirrus sac on one side, and from the dorsal to the ventral body-walls. The vas deferens, in addition to the tunica propria, possesses a delicate epithelial layer. As will be seen by comparison, the mass of the coils of the vas deferens in this species is much larger and more compact than in any form yet described. At all times in ripe proglottids the vas deferens is filled with mature spermatozoa in thick bunches. Just before it enters the cirrus pouch the duct decreases in size to a diameter of .015 mm., and the wall becomes thicker and acquires a layer of longitudinal muscles.

The cirrus pouch is roughly wedge-shaped, with the apex at its distal end. Its wall is .0035 mm. thick, composed of a longitudinal and a transverse muscle layer, and lined and covered with a delicate epithelium. It is intimately connected with surrounding tissue and never shifts its position. The wall of the sac extends distally to within .06 mm. of the body margin, then turns inward, and the longitudinal muscle layer is reflected back in the wall of the cirrus. It is in the action of these reflected muscles that the cirrus pouch aids the protrusion of the cirrus. When the cirrus is protruded the contents of the cirrus sac are fairly pulled away from its walls, which are never moved.

The vas deferens, where it enters the cirrus pouch, is .01 mm. in diameter, and has a wall .003 mm. thick, with faintly marked muscle fibers. Just within the pouch it is surrounded by a cluster of glandular cells. The duct slightly enlarges to

a diameter of .012 mm., while the walls gradually increase in thickness. Its course is a most intricate one, coiling and twisting, and not confined to any particular part of the pouch (Pl. XVI, Fig. 17). Its course is not identical in different proglottids, but all have much the same complicated twisting. At about the middle of the course of the vas deferens in the pouch a delicate cuticula, lining the cavity, makes its appearance; the longitudinal muscle fibers have become more prominent, forming a well-defined layer, and circular muscles are first plainly seen. Proceeding distally, the cuticula constantly but slowly increases until, at the male genital opening, it is continuous with the body cuticula, and of equal thickness.

The windings of the vas deferens are invested with a delicate structureless tissue, which is very elastic. It wraps the coils of the duct and holds them in a constant relation to each other, but is connected with the walls of the pouch only by delicate filaments.

The distinction between vas deferens and cirrus is simply a matter of size. The structure of vas deferens within the pouch in its distal half is as follows (Pl. XVI, Figs. 18 and 20): lining the lumen, which is about .015 mm. in diameter, is the cuticula, .002 mm. thick, and quite regularly cast into folds, like the bellows of a camera (Pl. XVI, Fig. 20, *ct.*). Without the cuticula is a very minute layer of transverse muscle fibers, which follows exactly the contour of the transverse folds (Pl. XVI, Fig. 20, *m.s.*). The longitudinal muscle layer, which lies next, is of unequal thickness, dipping down into the folds (Pl. XVI, Fig. 20, *mp.*). Above the muscle layers comes a loose layer, containing pyriform glandular cells, with narrow processes projecting down through the muscle layers, beyond which they could not be traced (Pl. XVI, Fig. 20, *cl.gl.*). Surrounding the entire duct is a delicate single layer of flattened epithelial cells with prominent nuclei (Pl. XVI, Fig. 20, *ep.*).

The last coil of the vas deferens brings its distal end back to the proximal end of the pouch near the ventral side. A sudden enlargement to .07 mm. in long diameter then takes place, and the duct bends upward and outward (Pl. XVI, Fig. 17). The cirrus begins at the sudden enlargement, which is slightly bulb-

shaped. The size increases very abruptly to .2 mm., then gradually narrows down to the genital opening, roughly conforming to the shape of the pouch (Pl. XVI, Fig. 17). At the largest part the cirrus in transverse section is .2 mm. in long diameter and .075 mm. in short diameter, being of elliptical shape. The cuticula is .005 mm. thick, and its folds are large and rounded. The circular muscle layer is .002 mm. thick, the longitudinal muscle layer is .0075 mm. thick, and where it dips into the folds increases to .015 mm. The layer of tissue next to the muscles is .02 mm. thick, and around this is the delicate epithelium. At the distal end of the cirrus the longitudinal muscle fibers reach nearly to the body cuticula and are inserted in the parenchyma. The greater part of these fibers, however, are reflected back in the longitudinal muscles of the pouch. That part of the cirrus which extends from the distal end of the pouch to the genital pore is a simple hollow stalk with very thick cuticula, and surrounded by the two muscle layers. The cirrus, when protruded, is cylindrical in section and longer than a proglottis (Pl. XVI, Fig. 4). In a transverse section of the extended cirrus, proceeding from without inward, the following layers will be found: a thick cuticula, circular and longitudinal muscles, a layer of gland-like cells, then a space, then an epithelial layer, glandular cells, longitudinal muscles, circular muscles, and finally the lining cuticula. By comparing this with a section of the retracted cirrus and with the vas deferens, it will be seen that the cirrus is evaginated and that the end which was proximal is now distal; while from the distal end runs back within the cirrus the vas deferens, with its layers in the normal position. By the action of the circular muscles the flat cirrus has become cylindrical, and the pull of the longitudinal muscles has turned it wrong side out. That portion of the cirrus which penetrated the body-wall is now seen as a muscular stalk (Pl. XVI, Fig. 5).

A cirrus somewhat resembling this has been described in *Diplobothrium simile* by Lönnberg ('92), being simply an invagination of the external cuticula, encircled by its muscle coats and connected with the vas deferens at its proximal end. Riggenbach ('96) describes in *P. fossata* a cirrus which closely



resembles that of *P. ambloplitis*, the chief difference being in the more delicate structure, and the lesser number of coils of the vas deferens within the pouch.

*Female Generative Organs.*—The opening of the vagina is directly anterior to the male opening and is of variable size. The duct passes directly inwards for about .05 mm., and then enlarges to twice the size of the opening. The lumen remains constant in diameter for about .35 mm., during which distance the tube curves anteriorly, and then posteriorly again, taking a course which varies from a nearly straight one to a semicircle, but which always ends at about the same level as the opening. Throughout the region just described the vagina possesses an extraordinarily developed sphincter muscle, which is .03 mm. in greatest thickness. This muscle is very compact and must possess great strength. It is by far the most powerful sphincter about the vagina yet described in any cestode. The absence of any hooks and the shape of the cirrus render this structure an important one in most of the *Proteocephali*. This sphincter makes the terminal region of the vagina nearly as large and prominent in sections as the cirrus pouch (Pl. XVI, Fig. 18, *va.*).

On emerging from the sphincter muscle the cavity of the vagina enlarges suddenly, forming an elongated chamber with convoluted walls, which is directly downward and backward, narrowing as it nears the median body line (Pl. XVI, Fig. 9, *va.*). From the opening to the end of this enlargement the vagina is lined by a continuation of the body cuticula, which becomes very thin in the chamber, and there bears delicate cilia (Pl. XVI, Fig. 18). Enclosing the sphincter is a layer of loose glandular cells resembling those of the cirrus (Pl. XVI, Fig. 19, *ms.* and *cl.gl.*). Outside of these cells is a delicate epithelial layer (Pl. XVI, Fig. 19, *ep.*). The chamber with convoluted walls is undoubtedly for receiving at the time of copulation a mass of spermatozoa, which may be passed on as the capacity of the remaining vaginal duct will permit. It is impossible to trace the transition from the lining cuticula to the free epithelial layer which lines that portion of the vagina extending from the chamber to the oviduct. This portion of the vagina

passes down the median line of the proglottis near the ventral side, forming a number of turns and coils (Pl. XVI, Fig. 9). The inner portion is slightly larger in diameter. The mass of the coils is just anterior to the ovaries, differing in this respect from other described species, in all of which the coils are further posterior. The end of this portion of the vagina is marked by a minute receptaculum seminis (Pl. XVI, Fig. 10, *r.sem.*). From this region the vagina continues as a heavy-walled duct of decreased diameter, supplied with a layer of gland cells, and more heavily ciliated than before. This heavy-walled portion of the vagina by a series of curves reaches nearly to the posterior end of the proglottis, and there unites with the oviduct (Pl. XVI, Fig. 10). Further investigation will probably show that in *Proteocephalus* the vagina usually opens into the oviduct and not into the oötype. Such is undoubtedly the case in *P. filicollis*, although Kraemer ('92) describes the vagina as entering the oötype. *P. abscissa* and *P. fossata* Riggenbach ('96) both show the former condition.

The presence of spermatozoa can be plainly seen in most specimens; in some cases the vaginal chamber near the sphincter will be packed with them; other specimens show this portion with walls collapsed and lumen empty, while the coils near the ovary are distended.

The ovary lies in the posterior end of the proglottis, close to the ventral surface. It is a bi-lobed organ, each half extending from the median line, where they are joined together, to the vitellarian glands on the side. Each half is somewhat retort-shaped, with the small end inward, whether viewed in frontal or transverse section (Pl. XVI, Fig. 9, *o.*).

The ovary is surrounded by a delicate epithelial layer; the ova are closely packed together, those at the extreme outer end being clearly less developed than those toward the center. The ova are .01 mm. in diameter, with a nucleus measuring .005 mm., and containing a very prominent nucleolus. The ovary is penetrated by a few delicate sheets of tissue, which divide the eggs into irregular groups, extending from dorsal to ventral surface, but all open at the inner end, thus leaving the passage free into the oviduct. The two lateral wings of the ovary are

joined at their inner extremities by a portion of varying size, which is antero-dorsal to the rest of the ovary. From this common portion projects a muscular organ, the oöcapt,<sup>1</sup> commonly known as the "Schluckapparat" of the German investigators, which marks the beginning of the oviduct (Pl. XVI, Fig. 6). This organ is of oval shape, .025 mm. wide, and .03 mm. long. It is composed of a layer of circular muscles, .008 mm. thick, surrounding a lumen, .0075 mm. in diameter, which is lined by cubical epithelial cells. The contractions of this organ force the eggs forward into the empty oviduct, as the diameter of the outer end of the lumen is .002 mm. greater than that next to the ovary. About the circular muscles is a coat of fibrous tissue, .007 mm. thick, in which, at fairly regular distances, are long, spindle-shaped glandular cells, with narrow processes running toward the lumen.

The oöcapt has been described in *P. filicollis* by Kraemer ('92), in *P. coryphicephala* by Monticelli, in *P. longicollis* by von Linstow ('91), and in *Corallobothrium lobosum* by Riggenbach ('96). The oviduct emerging from the oöcapt is .015 mm. in diameter. It is lined by cubical epithelial, ciliated cells, and surrounded by loose tissue containing gland-like cells.

The course of the oviduct differs from that of species hitherto described in that it never passes directly backward into the oötype, but always passes toward the posterior margin of the proglottis, and then curves forward into the oötype (Pl. XVI, Fig. 10, *od.*). Starting from the oöcapt it makes a wide curve laterally, then turns to the ventral side close to the margin of one of the ovarian lobes, thence follows a wide sweep towards the opposite lobe of ovary, from whence it curves forward and

<sup>1</sup> To my knowledge there exists in English no name for this structure which is of such importance and general occurrence in the cestodes. Some authors have recently endeavored to transfer the German name directly into our scientific terminology. The unsatisfactory character of such usage needs no demonstration here. I am accustomed to use the form given in the text to designate this organ. The name is derived from *ὄον*, egg, and *κάρως*, the gulper or swallower, being thus analogous to the German term, and of similar formation to the designation oötype, used for an associated organ. Since the name has never appeared in print, this formal statement of its derivation and meaning seemed advisable.



upward, passing into the oötype about the center of that space. Its course is never exactly the same in different proglottids. Throughout its course the oviduct is supplied with gland-like cells, enclosed in a single-layered epithelium and lined by a layer of ciliated epithelial cells (Pl. XVI, Fig. 6). The oviduct is easily distinguished from other ducts in this region by reason of its greater size, darker staining, and more regular course (Pl. XVI, Fig. 16, *od.*). The diameter remains about the same throughout. The ova, as they pass down the oviduct, are fertilized by the spermatozoa from the vagina before they reach the oötype.

The oötype is a direct continuation of the oviduct (Pl. XVI, Fig. 12, *ot.* and *od.*). It possesses a circular muscle layer, and for its entire length is surrounded by the shell gland. Its position is about the center of the interovarial space. It receives the common vitellarian duct at its beginning, and the secretion of the surrounding shell gland throughout its course, resembling precisely the oötype of *P. torulosa* and *P. filicollis* (Kraemer, '92). It has exactly the same structure, with the addition of a muscular coat, as has the oviduct, even to being provided with the gland cells, which are embedded in the shell gland (Pl. XVI, Fig. 12). Its entire length is only .06 mm., and its diameter .015 mm. Its walls are no thicker than those of the oviduct, the cubical epithelial cells not being so large, and this decrease being equalized by the muscle layer. The oötype opens directly into the uterus, there being here no intermediate duct, such as is commonly described in other species. It is possible that one of the coils of the thick-walled portion of the vagina has been mistaken in some cases, at least, for the duct leading to the uterus. There can be no doubt in *P. ambloplitis* that the uterus extends directly forward from the oötype, yet many sections seem to show such a duct, which, on careful reconstruction, always proves to be nothing more than one of the coils of the vagina. The walls of the uterus are very delicate, of a fibrous texture, and the lumen at its beginning is .05 mm. in diameter. It runs directly antieriad to just in front of the ovary, where it turns back and proceeds in a dorso-posterior direction to near the posterior end of the proglottis. It then makes a turn toward

the anterior, and spreads out in all directions, increasing in size with the development of the proglottis. The uterus spreads first over the dorsal portion of the space, which contains the sexual organs, and when fully developed occupies nearly this entire space, with the exception of the rudiments of other sexual organs. The branches of the uterus are few, and variable in size and form. The tissue remaining is in the form of thin partitions, which stand out perpendicularly from the side.

The shell gland is more compact than that of most of the *Proteocephali*, and extends equally on all sides of the oötype. It appears as an irregular granular mass, with scattered nuclei distinguishable within it, and large glandular cells grouped irregularly in the outer portion. No trace of collecting tubules can be distinguished, and the fibrous appearance indicates a process to the oötype from each cell. This shell gland is much larger and more compact than that described in any other species of the genus (Pl. XVI, Fig. 12).

The vitellarian ducts occupy the lateral margins of the proglottids, just within the longitudinal muscles and partly encircling the nerve (Pl. XVI, Fig. 9, *vt.gl.*). Two large ducts run from the anterior portion toward the posterior end of the proglottis. Numerous branches of various sizes join them in their course. About the level of the anterior margin of the ovary the main duct on each side curves inward and passes along the dorsal wall until it is over the oötype; here the two unite to form the first vitellarian receptacle (Pl. XVI, Fig. 10, *vt.r.*). Such a union of the yolk ducts from the two sides seems to be a characteristic of the genus *Proteocephalus*. Riegenbach ('96) describes such a condition in the forms he has studied, and says that only in *P. filicollis* do the ducts enter the oötype separately. *P. filicollis*, however, is *not* an exception, as my own work has proved that the ducts unite exactly as in *P. ambloplitis* (see p. 364).

There are many variations in size, and some in the number of these ducts; there may be two from one side and only one from the other, or those from one side may be very large, while the others are narrow. The yolk cells are spherical masses containing small, densely staining globules.

From the first vitellarian receptacle a duct runs ventrally and expands into a prominent second yolk reservoir (Pl. XVI, Fig. 12, *vt.r.*), which is situated close to the beginning of the oötype. From this reservoir, which is larger than the first, a narrow, heavy-walled duct leads into the oötype.

As the proglottis nears maturity the dermo-muscular sac begins to get thinner for a short distance in the mid-dorsal line. As the uterus becomes distended with eggs, it pushes up into this portion. With the further growth of the uterus and development of the eggs, the body-wall at this point becomes thinner and thinner, until it breaks apart and a cleft is formed. Then the pressure on the wall of the uterus at this point causes its rupture and the eggs escape.

*Proteocephalus filicollis* Rud.

The early investigators, Rudolphi ('10), Dujardin ('45), Diesing ('50), and von Linstow ('78), all described *Taenia filicollis* and *Taenia ocellata* as distinct species, as also did Zschokke ('84). The last author, however, added something of the anatomical structure to the details of size and external form given by the older writers. Kraemer ('92) was the first to study by the section method these two forms, making an exhaustive anatomical and histological investigation, which showed *T. filicollis* and *T. ocellata* to be identical. As *T. filicollis* has the precedence in Rudolphi's synopsis, this name should always apply. Riggenbach ('96) unaccountably states that *ocellata* has the precedence, although *T. filicollis* is described in Rudolphi's work ('10) on p. 106, and *T. ocellata* on p. 108.

In *P. filicollis* Kraemer established the presence of a nervous mass in the scolex, and two longitudinal nerve trunks through the body. The excretory system was shown to consist of a network of ducts in the scolex and neck, and four longitudinal vessels through the proglottids. He demonstrated the similarity of the cuticula with that of other cestodes. The vagina was shown to open in front of the male opening, and to possess at its distal end a sphincter muscle. He established the presence of a bi-lobed ovary in the posterior end of the proglottis and

of an oviduct leading from it to an oötype — a duct with a muscular coat. The lateral position of the vitellaria, the elongated acinous structure, and the ducts which proceeded from them to the oötype were described. The uterus was shown to be a thin-walled sac with lateral branches in the median line of the proglottis. A shell gland was demonstrated surrounding the oötype. He pointed out the number and scattered position of the testes, and the ducts which collected the spermatozoa. He also showed that these ducts unite to form the vas deferens, which is coiled in the center of the proglottis, and functions as a seminal vesicle. Finally he described the termination of the vas deferens in a muscular cirrus, enclosed in a well-developed cirrus pouch. These characteristics show it to be a typical species of *Proteocephalus*.

The specimens which I received from Dr. Ward's collection were in four lots, two of these having been taken from *Coregonus nigripinnis*, one from *C. prognathus*, and one from *C. artedi*. They were obtained by Dr. Ward while in charge of a biological examination of Lake Michigan, under the auspices of the Michigan Fish Commission, during the summer of 1894. All of the fish were caught in the Traverse Bay region, near Charlevoix, Mich. The cestodes were found in the stomach of *C. artedi*, and of one specimen of *C. nigripinnis*, and in the intestine of *C. prognathus* and of the other specimen of *C. nigripinnis*.

#### *General External Morphology.*

In external form those from *C. artedi* and *C. prognathus* were alike (Pl. XVI, Fig. 21), while those taken from *C. nigripinnis* were longer than the first, and the neck was not only much longer, but also slenderer (Pl. XVI, Fig. 22). In the shorter specimens the length varied from 12 mm. to 16 mm., while the breadth at the widest point was .8 mm., and the neck was about two-fifths the total length of the body. In the other form the length varied from 15 mm. to 25 mm., the breadth was .8 mm., while the neck ranged from three-fifths to four-fifths of the entire body length. In one of the two lots which came from *C. nigripinnis* was a specimen very much larger than the others.













Its total length was 38 mm., the length of the neck was 7 mm., but the body was no broader than the others. This specimen corresponds more closely to Kraemer's description than the others, both as to total length of body and as to the comparative length of the neck. The fact that the above forms are identical in all other respects shows how little importance can be attached to size alone as a means of identification.<sup>1</sup>

The scolex is .12 mm. in diameter, being of a somewhat globular shape. It resembles, under low magnification, a rounded, slightly swollen end of the neck. The four suckers are .04 mm. in diameter, directed outward and upward, and situated a little above the equatorial plane of the scolex (Pl. XVI, Fig. 24).

The fifth sucker is in every respect a true sucker, since it has the same structure and musculature that the four large suckers possess. The whole region of the scolex anterior to the suckers is capable of considerable motion. Some specimens show the whole region retracted until it is "dished" below the anterior margin of the suckers. In another it may be very prominent, with the opening hardly visible. The scolex is flattened dorso-ventrally, and slight furrows separate the suckers.

The neck is .10 mm. to .12 mm. broad at its anterior extremity, and varies from 5 mm. to 10 mm. in length. The first proglottis is very faintly marked off from the neck, and in most cases is longer than wide; in a few specimens, however, the first proglottis is nearly square. This proglottis varies in breadth from .12 mm. to .20 mm., and in length from .12 mm. to .16 mm. The total number of proglottids ranges from nine to twenty, though the single very large specimen spoken of had forty. The sexually mature proglottids come about the middle of the chain. These vary in number from four to seven, and are not

<sup>1</sup> The many differences which I found between *P. filicollis*, as described by Kraemer, and my own specimens led me to doubt the accuracy of their identification. Through the kindness of Professor Zschokke, Dr. Ward obtained some specimens of the true *P. filicollis*, which he permitted me to examine. Unfortunately, however, during the passage the alcohol had escaped from the vials, and the specimens arrived in a dry state. The most careful treatment only partially restored them, but an investigation convinced me that they resembled my own specimens too closely to justify me in founding a new species.

identical in shape in different individuals, varying in form from nearly square to an oblong in which the length is twice the breadth. The greatest breadth of the body comes in the next to the last proglottis. The posterior proglottis makes a gradual taper toward the posterior end, which is smoothly rounded. The excretory pore can be seen at the tip as a cup-shaped indentation.

*Anatomical and Histological Structure.*

*Cuticula.* — The cuticula is very delicate, .0035 mm. in thickness; the external layer presents a very rough appearance (Pl. XVI, Fig. 29, *ct'*). It takes stain more deeply than the other layer. There is no smooth outline on the exterior, as drawn by Kraemer. What he pictures as a homogeneous layer, penetrated by perpendicular canals, is in reality a ragged, exceedingly irregular layer, with clefts and breaks reaching down to the next layer. Its appearance suggests the sloughing off of the cuticula. The layer of cuticula beneath this is .002 mm. thick, and stains lightly (Pl. XVI, Fig. 29, *ct*).

Beneath the cuticula is a layer of circular muscles .001 mm. thick, and within these is a layer of longitudinal muscles, which in cross-sections shows as a row of dots between the outer ends of the cell processes of the next layer (Pl. XVI, Fig. 29, *m.l.ct.* and *m.c.ct.*).

The subcuticular cell layer is very prominent, having a thickness of .025 mm. (Pl. XVI, Fig. 29, *sb.c.*). The cells are narrow, fusiform, with long delicate processes on the distal ends. The subcuticular cell layer in *P. filicollis* is much more prominent than in *P. ambloplitis*. In transverse sections the cells form a band, over half the thickness of the dermo-muscular sac in *P. filicollis*, while in *P. ambloplitis* the band only constitutes one-fifth of the thickness of the dermo-muscular sac (Pl. XVI, Figs. 16 and 23, *sb.c.*).

The parenchyma is very open and loose in texture, looking like a coarse, irregular meshwork. Muscular fibers are scattered throughout it, extending in a transverse direction. In the area enclosed by the longitudinal body muscles the parenchyma

presents the appearance of strands interwoven, being still more open in texture than the outer parenchyma.

Calcareous bodies are not abundant and are very small, none being longer than .005 mm.

*Nervous System.*—The central nervous system consists of a ring a little anterior to the middle of the scolex; a large short trunk runs from the median dorsal, the median ventral, and two lateral points of the ring about half the distance to the cuticula, where it divides and half goes to each adjacent sucker (Pl. XVI, Fig. 25, *n.r.* and *n.*). From the central nerve ring two longitudinal trunks pass down the body just within the longitudinal muscle bundles at the sides. The nervous systems of *P. filicollis* and *P. ambloplitis* are thus almost exactly identical.

*Excretory System.*—There are four regular longitudinal canals traversing the body. The walls of these ducts are much thicker than in *P. ambloplitis*, and the course of the duct is more regular, with fewer branches. Branches of various sizes are given off at irregular intervals and take a more or less tortuous course among the organs. The branches which extend toward the cuticula are straighter, give off fewer branchlets, finally becoming lost in the subcuticular layer. In some cases the ducts seem simply to unite with parenchyma spaces and can be traced no further. In the cuticula, at rare intervals, an opening may be found through which the excretory system connects with the exterior. These openings are neither so large, so numerous, nor so well defined as in *P. ambloplitis*. In the scolex and anterior portion of the neck the four vessels are much branched, the branches forming a network much as in the latter species. Yet the network so formed is not so complex as in *P. torulosa*.

*Musculature.*—The musculature is not so strongly developed as in *P. ambloplitis*. The longitudinal muscles are arranged in numerous small bundles, appearing in transverse sections of proglottids as irregular rows of dots parallel to the outline of the section (Pl. XVI, Fig. 23, *m.l.*). The sections of the bundles are of irregular shape and vary in breadth from .0015 mm. to .004 mm.



A loose sheet of circular muscle fibers weaves around the longitudinal bundles. Large fibers pass in a transverse direction between these muscle sheets. The divisions between the proglottids are formed by the interlacing of these fibers with similar ones which cross them at right angles, both sets being here much more complicated than in other regions.

In the scolex the longitudinal muscles are represented by scattered fibers, not arranged in compact bundles, passing anteriorly between the suckers, a few extending to the anterior part of the scolex. Other fibers, arranged in a radial direction, extend between the suckers, and are connected with the musculature of these organs.

*Sexual Organs.*—A common genital sinus is present, situated a little anterior to the middle of the margin of the proglottis. Its position is irregularly alternate in the chain of proglottids.

*Male Organs.*—The testes are from thirty-five to fifty in number, and about .05 mm. in diameter. They are scattered throughout the space between the longitudinal muscles and the vitellaria. Each testis is closely invested by the tunica propria. Minute ducts collect the spermatozoa and join together, making larger ducts near the center of the proglottis. These larger ducts unite to form a dilated thin-walled vas deferens .02 mm. in diameter, which forms a convoluted mass of coils in the middle of the proglottis (Pl. XVI, Fig. 27, *v d.*). The wall of the vas deferens consists of the tunica propria and an epithelial covering, while the coils are closely bound together by parenchymal strands. The vas deferens is always distended with spermatozoa.

The distal end of the duct passes upward to the dorsal muscle bundles, and there enters the cirrus sac. At a distance of .10 mm. from its entrance to the cirrus it undergoes a sudden diminution in size and receives a delicate muscular coat as well as an epithelial lining. At the point where the vas deferens enters the cirrus sac it is only .015 mm. in diameter, with walls .005 mm. thick, the greater part of which is a muscular coat. The duct bends sharply as it enters the cirrus pouch.

The cirrus sac is an elongated oval in shape, slightly constricted in the middle. It extends from the edges of the geni-



tal pore to the median dorsal line of the inner parenchymal space, where a number of heavy muscle strands attach the proximal end of the inner tube of the pouch firmly to the dermo-muscular sac. The walls are thickest about the middle of its length, and consist of a circular and transverse muscle layer, with a delicate epithelium covering the outer and inner surface (Pl. XVI, Fig. 34).

At the anterior end the wall of the pouch bends back, forming a tube through the middle of the sac (Pl. XVI, Fig. 31). This tube is lined, in the distal half, by cuticula, which is continuous with that covering the surface of the body. At the posterior end no cuticula can be distinguished, and the muscle layer is very thick. The space between the inner and outer walls of the cirrus sac is filled with fibrous tissue, with many nuclei (Fig. 31). The fibers run from wall to wall in transverse direction toward the proximal end, while near the distal portion the fibers are curved, with the concave portion directed proximally. At the posterior end of the inner tube the thick muscle layer sends out large strands, which pass out of the proximal end of the cirrus pouch and join the dermo-muscular sac, as before stated.

About the tube, within the sac, are many gland cells, projecting perpendicularly from its walls, and most numerous in its proximal half.

The cirrus is straight, cylindrical, and of approximately equal diameter throughout, with the exception of the distal termination (Fig. 31, *ci.*). This portion is a muscular knob .02 mm. in greatest diameter, composed principally of circular muscles. In its normal position this terminal portion rests in the end of the inner sac tube, like a ball on a cup. The sides of the distal fourth of the cirrus are not in direct contact with the cuticula lining the inner tube, and throughout this distance the wall of the tube is quite thick, .005 mm., and the cuticula is covered with fine, stiff, bristle-like projections. The cirrus has a lumen of .01 mm., which is lined by a thin epithelial layer. The organ here presents a very peculiar appearance, due to the circular muscles which compose its walls, and which are here .004 mm. thick. Throughout most of its course the cirrus is

very closely joined to the walls of the inner tube, and it seems probable that it does not move through the tube at all.

Kraemer's description is widely different. His drawing shows a slender tube with hooks, representing the cirrus, and followed by a proglottis-like arrangement, and then several coils of the vas deferens within the pouch. The drawing has an unreal appearance on the first glance. The cirrus, with its curved hooks, is imbedded in the tissue, which must be torn through before it could be protruded. The proglottis-like appearance of a portion of the cirrus, as he drew it, was undoubtedly due to the way in which the circular muscles were cut in sectioning (Pl. XVI, Fig. 31, *m.s.*). He drew the cavity of the cirrus, into which the cut ends of the circular muscles projected, as the external outline of the cirrus. The coils would be necessary, according to his theory, but are not to be found. He says that the muscles, which he calls "the roots," are for retracting the pouch, which is sometimes thrust outwards for some distance through the opening. This protrusion is really due to an evagination of the free distal end of the inner tube.

When the cirrus is entirely protruded it extends a length of .10 mm. from the male opening. The distal end is the muscular knob at the end of the free part of the cirrus, which is .035 mm. long. At the proximal end this free cirrus enters a muscular protuberance of the shape of a truncated cone, whose exterior is covered by minute, bristle-like structures. The cirrus passes through the center of this and back through the pouch in very intimate connection with the walls of the inner tube of the sac. The mechanism of the protrusion seems to be as follows: The longitudinal muscles of the cirrus sac, which are reflected at the distal end of the pouch into the wall of the inner tube, by contraction, would evaginate that distal portion of the tube which is free from the cirrus, and pull the rest of the inner tube outward through the sac, stretching the "roots" (Pl. XVI, Fig. 32). Because of the close connection between the walls of the cirrus and those of the inner tube, the latter would carry out with it the cirrus (Fig. 32). The evaginated distal portion of the tube forms the muscular protuberance, the former rough cuticular lining of which is now on the outside.

Retraction would be accomplished principally by the roots, which, as has been seen, are attached to the proximal end of the inner tube. The vas deferens for a short distance without the cirrus sac is of the same size as the cirrus itself, and could be drawn into the pouch, thus providing for the extension.

*Female Generative Organs.*—The vaginal opening is .008 mm. in diameter. About .005 mm. back of the opening, a sphincter muscle is found, .008 mm. in thickness and hemispherical in cross-section. The vagina then bends backward and inward, passing under the middle of the cirrus sac. It is of nearly equal size throughout, and is lined by ciliated epithelial cells. Outside of this is a thin, circular muscle layer bounded by delicate epithelium. The whole vagina, toward its distal extremity, is surrounded by a very prominent layer of glandular cells, with long processes extending toward the duct. The vagina follows the median line of the proglottis, close to the ventral body muscles. Its course continues straight until it passes under the ovaries, where it forms a number of coils; from there passing to the posterior end of the proglottis, and opening into the oviduct (at *od.*, Pl. XVI, Fig. 28). Kraemer ('92) wrongly states that it opens into the oötype.

The ovary is a bi-lobed organ, composed of two retort-shaped parts, connected in the median line by a narrow portion, corresponding to the neck of the retort (Fig. 28, *o.*). Each half of the ovary is covered by a delicate membrane, composed of a single layer of cells. The ovary is closely packed with ova, which are .01 mm. in diameter when they are ready to leave the organ. The germinal vesicle is very large, measuring .0075 mm. in the mature ova, with a prominent nucleolus .005 mm. in diameter. The eggs which are found in the uterus have a diameter of .02 mm. This last measurement agrees with that of Kraemer, but it will be noticed that the immature ova in this specimen seem to be nearly twice the size he describes. This difference might be accounted for by his overlooking the delicate protoplasmic mass, which forms the outer portion of an ovum. The ova pass from the ovary into the oöcapt, which is .025 mm. long and .02 mm. in diameter. It is lined by cubical epithelium, which is surrounded by a muscu-

lar band, oval in section (Pl. XVI, Fig. 35). This organ in *P. filicollis* resembles closely that of *P. ambloplitis*.

The oviduct is lined by cubical, thickly ciliated cells. Around this layer is a thin stratum of what appear to be muscle elements running transversely; then comes an epithelial covering, and outside of this are scattered gland cells. The course of the oviduct depends somewhat on the direction of the oöcapt, requiring another curve if this organ is directed antieriad; but in all cases the oviduct passes in slightly curving lines nearly to the posterior margin of the proglottis. Here it may make a coil or two, or bend directly forward again to the center of the inter-ovarial space. The vagina usually enters the oviduct near the posterior margin of the proglottis, although the union is not at exactly the same place in different proglottids.

The oötype seems to be simply a specialized portion of the oviduct. The muscle layer is a little thicker; the cilia are very small, or entirely lacking; and the surrounding gland cells are more numerous. The organ is .0125 mm. long, and for the entire distance is surrounded by the shell gland, which in this form is exceedingly irregular in shape. Around the oötype is a dense portion with a striated appearance, as if composed mainly of cell processes (Pl. XVI, Fig. 30, *sh.gl.*). Long strands extend outward from here, leading to bunches, or masses, of cells of irregular shape. There is, moreover, no similarity in the form of the shell gland in different proglottids.

The vitellaria are found on the lateral margins of the space within the dermo-muscular sac. Each is formed of a loose mass of vesicles, joined to a central longitudinal duct by fine transverse branches (Pl. XVI, Fig. 27, *vt.gl.*). The central ducts at the base turn directly inward, and meet a little anterior to and above the oötype. At their junction a small vitellarian reservoir is formed of variable size. From this reservoir a narrow duct leads downwards and backwards to a larger spherical reservoir, lying just above the oötype, into which it empties by a very narrow duct (Pl. XVI, Fig. 30, *vt.r.*).

The uterus extends as a delicate walled tube anteriorly from the end of the oötype. It passes forward under the connecting



portion of the ovaries, then turns upward and spreads out in a broad sheet, close to the longitudinal muscles above the testes. Lateral outpocketings are formed as the proglottis becomes more mature, and these branches grow larger until the uterus in the posterior proglottids nearly fills the entire space within the dermo-muscular sac (Pl. XVI, Fig. 27, *ut.*).

The ova escape by a cleft in the mid-dorsal wall of the proglottids. The formation of the cleft is very gradual and seems to result from a gradual thinning of the dermo-muscular sac at that place.

In the relations of glands and ducts *P. filicollis* and *P. ambloplitis* are remarkably similar.

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## EXPLANATION OF PLATE XVI.

The figures are drawn at the scale indicated and, unless otherwise stated, with the Abbé camera lucida. Sections from which drawings were made were stained with haematoxylin.

## REFERENCE LETTERS.

<i>ca.</i>	calcareous bodies.	<i>o.</i>	ovary.
<i>ci.</i>	cirrus.	<i>od.</i>	oviduct.
<i>c.m.</i>	circular body muscles.	<i>oc.</i>	oöcapt.
<i>c.p.</i>	cirrus pouch.	<i>ot.</i>	oötype.
<i>ct.</i>	external cuticular layer.	<i>pa.</i>	parenchyma.
<i>ct.</i>	internal cuticular layer.	<i>p.ex.</i>	excretory pore.
<i>cl.gl.</i>	gland cells.	<i>sb.c.</i>	subcuticular cells.
<i>d.ex.</i>	excretory ducts.	<i>sh.gl.</i>	shell gland.
<i>ep.</i>	epithelial layer.	<i>sp.va.</i>	sphincter of vagina.
<i>ep.cil.</i>	ciliated epithelium.	<i>t.</i>	testes.
<i>l.m.</i>	longitudinal body muscles.	<i>tr.m.</i>	transverse muscles.
<i>m.c.ct.</i>	circular subcuticular muscles.	<i>ut.</i>	uterus.
<i>m.l.ct.</i>	longitudinal subcuticular muscles.	<i>v.d.</i>	vas deferens.
<i>ms.</i>	circular muscles.	<i>va.</i>	vagina.
<i>mp.</i>	longitudinal muscles.	<i>vt.d.</i>	vitellarian duct.
<i>n.</i>	nerve.	<i>vt.gl.</i>	vitelline gland.
<i>n.r.</i>	nerve ring.	<i>vt.r.</i>	vitelline reservoir.

FIGS. 1-20. *Proteocephalus ambloplitis*.

- FIG. 1. Lateral view of scolex.  $\times 25$ .  
 FIG. 2. Anterior view of scolex.  $\times 25$ .  
 FIG. 3. Median sagittal section of scolex.  $\times 50$ .  
 FIG. 4. Proglottids showing extruded cirrus.  $\times 25$ .  
 FIG. 5. Proximal portion of extruded cirrus.  $\times 100$ .  
 FIG. 6. Oöcapt and beginning of oviduct.  $\times 500$ .  
 FIG. 7. Dorsal view of scolex, showing portion of nerve ring and the longitudinal nerve trunks.  
 FIG. 8. Transverse section showing nerve ring with branches to suckers.  
 FIG. 9. Reconstruction of frontal sections showing the organs in a proglottis. Camera outlines.  $\times 50$ .  
 FIG. 10. The intra-ovarial region with enclosed female organs.  $\times 100$ .  
 FIG. 11. Section through cuticula and subcuticular structure.  $\times 2000$ . Not a camera drawing.  
 FIG. 12. Section through oötype, shell gland, and vitelline reservoir.  $\times 250$ .  
 FIG. 13. Termination of longitudinal excretory ducts in the posterior proglottis.  $\times 25$ . Not a camera drawing.  
 FIG. 14. Transverse section of very young proglottis, showing the eight longitudinal excretory ducts.  $\times 25$ . Not a camera drawing.

- FIG. 15. Longitudinal dorsal excretory vessels.  $\times 25$ .  
 FIG. 16. Transverse section near posterior end of proglottis.  $\times 50$ .  
 FIG. 17. Course of vas deferens and cirrus within the cirrus sac. Reconstruction from camera outlines.  $\times 250$ .  
 FIG. 18. Frontal section through cirrus pouch and vaginal opening.  $\times 100$ .  
 FIG. 19. Transverse section through vaginal sphincter.  $\times 200$ . Not a camera drawing.  
 FIG. 20. Transverse section of vas deferens at its distal end.  $\times 1000$ . Not a camera drawing.

FIGS. 21-35. *Proteocephalus filicollis*.

- FIG. 21. Outline of short form of *P. filicollis*.  $\times 4$ .  
 FIG. 22. Outline of long form of *P. filicollis*.  $\times 4$ .  
 FIG. 23. Transverse section of proglottis through ovarian region.  $\times 100$ .  
 FIG. 24. Lateral view of scolex.  $\times 200$ .  
 FIG. 25. Transverse section of scolex showing nerve ring.  $\times 200$ . Not a camera drawing.  
 FIG. 26. Median frontal section of scolex showing nerve trunks.  $\times 200$ . Not a camera drawing.  
 FIG. 27. Reconstruction of frontal section showing anatomy. Camera outlines.  $\times 75$ .  
 FIG. 28. Female sexual organs of the intra-ovarian space.  $\times 225$ .  
 FIG. 29. Section through cuticula and subcuticular structures.  $\times 2500$ . Not a camera drawing.  
 FIG. 30. Section of oötype, shell gland, and vitelline reservoir.  $\times 500$ .  
 FIG. 31. Cirrus pouch and cirrus in retracted condition.  $\times 200$ .  
 FIG. 32. Cirrus pouch with cirrus protruded.  $\times 200$ .  
 FIG. 33. Frontal section through distal end of vagina.  $\times 200$ .  
 FIG. 34. Transverse section through the middle of the cirrus pouch.  $\times 600$ . Not a camera drawing.  
 FIG. 35. Section of oöcapt and proximal end of oviduct.  $\times 250$ .















# THE EARLY DEVELOPMENT OF PLANORBIS.

SAMUEL J. HOLMES.

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THE investigation, the results of which are recorded in the present paper, was carried on under the supervision of Prof. C. O. Whitman, at the University of Chicago and at the Marine Biological Laboratory at Woods Holl, Mass. It is a source of gratification to acknowledge the generous treatment I have received in both these places at Professor Whitman's hands. I wish also to express my appreciation of the many suggestions I have received from Dr. E. G. Conklin, both in person and through his admirable paper on the "Embryology of *Crepidula*."

The species studied is *Planorbis trivolvis* Say. This species occurs in great abundance in the ponds of South Park, Chicago, and it was also found in a pond near Falmouth, a few miles from Woods Holl.

## PART I. DESCRIPTIVE PORTION.

### *Methods.*

The eggs of *Planorbis*, when brought into contact with water, swell quite rapidly, so it is best to tease them out of the capsules directly into fixing fluid. The eggs may, however, be teased first into normal salt solution, to which it is better to add a small quantity of the fixative, and then treated with the fixing fluid alone. Some fixing fluids coagulate the albuminous substance around the egg so quickly, after or during its escape from the capsule, that it usually becomes surrounded with more or less coagulated albumen, from which it is difficult to free it. By teasing directly into normal salt solution, mixed with only a small quantity of the fixative, the eggs may be obtained free from any foreign material. Normal salt alone often causes more or less swelling.

Formalin does not appear to coagulate the albuminous substances in the capsules. The egg masses may be kept in a 5% solution of this substance for several days, the jelly remaining perfectly fluid and transparent. Unfortunately, formalin does not otherwise prove a satisfactory fixing agent. Kleinenberg's stronger picro-sulphuric gave good results, especially when followed by the method of staining with acidified Delafield's

haematoxylin, used by Conklin. Lithium carmine proved a good nuclear stain when the eggs were first overstained, and the color extracted by a long treatment with acid alcohol. Haematoxylin has the disadvantage, for the later stages of cleavage, of staining very intensely the small globules of albuminous material, which become very numerous and render the nuclei difficult to observe.

By far the most useful reagent that was employed was silver nitrate. In fact, were it not for the beautiful and clear preparations obtained by using this stain, I should probably have been unable to follow the cell lineage of *Planorbis* to the stages here described. The eggs are teased from the capsules directly into a .75% solution of silver nitrate, and exposed in a watch glass to the sunlight, the brighter the better. The eggs may be examined from time to time with the microscope, and when the cell boundaries stand out clearly, the nitrate is removed and the eggs washed quickly in water. The water being mostly removed, a few drops of a  $\frac{1}{5}$ % solution of hyposulphite of soda is added and allowed to act only three or four seconds. In fact, I begin to remove the hyposulphite as soon as it is poured on the eggs. The object of this treatment is to prevent after-blackening of the eggs by dissolving out the unreduced silver. Otherwise the eggs are liable to become in time so dark that they are useless for study. A too prolonged treatment with the hyposulphite, on the other hand, will destroy the silver stain entirely. I have found it unsafe to wash the hyposulphite out with water. The eggs often suddenly swell to twice their normal volume and are thereby spoiled. Instead of water, a saturated solution of picric acid may be used. This acts at the same time as a fixative and does not injure the stain. After a few minutes' treatment with this reagent, the eggs may be passed through the grades of alcohol, cleared in xylol, and mounted in balsam.

I have used to support the cover glass strips of paper of the proper thickness gummed to the slide. By moving the cover, the eggs may be rolled so that they can be studied from all sides. When the balsam becomes hard, it can readily be softened by applying a drop or two of xylol to the edge of the cover glass.

Eggs treated by the foregoing method will keep indefinitely, neither fading nor becoming opaque. When the egg is mounted, the stain is usually safe. In successful preparations the cells of the egg are not strongly darkened, but the cell boundaries stand out in a remarkably sharp and clear manner. Some cells, however, stain much darker than others. The trochoblasts and the cells of the head vesicle remain almost perfectly transparent, while the cells of the cross are colored brown. Owing to the transparency of the trochoblasts the cross appears with a wonderful distinctness, enabling one to orient the egg at a glance. Nuclei are not stained, but they can usually be seen, and the spindles of dividing cells are often visible. The eggs may, however, be stained so that the nuclei show fairly well, but I have generally dispensed with nuclear staining when using this method. The treatment often injures the impregnation and renders the egg more opaque, so that it is more often a nuisance than a benefit. Individual differences in the impregnation of different eggs are quite decided. Even among eggs from the same capsule subjected to exactly the same treatment, some will be strongly stained, while the staining in others is faint. If eggs are left too long in the nitrate, they not only become too dark, but also become brittle, and the cells break or become separated when the eggs are rolled. Cell boundaries take the stain at all periods in the development of the egg, even in the very first cleavage stages. The method is of special value, however, in following the cell lineage of organs in the later periods of cleavage. I have obtained wonderfully clear preparations of gastrulae, which show the boundaries of each cell, when there are several hundred cells in the egg, with diagrammatic distinctness. The cells of the proto-troch in the gastrula stage form a conspicuous transparent band, which appears in marked contrast to the adjacent cells—a circumstance which proved very helpful in tracing out the cell lineage of this organ.



*Nomenclature.*

In the matter of nomenclature I have followed the system used by Conklin in his paper on the "Embryology of *Crepidula*," as this method enables one readily to follow the type of cleavage found in annelids and mollusks. Besides, the comparisons constantly to be made throughout this paper, with the work of Dr. Conklin, render it highly desirable, aside from other considerations, that the same system of nomenclature be employed. The word "quartette" is used to designate the products of a generation of cells given off from the four cells at the vegetal pole of the egg. The term "quartette" has been used, however, in a different sense by several writers, who employ it to designate any four cells of radially symmetrical origin. Thus, according to the latter usage, the four outer cells arising from the division of the first generation of ectomeres would constitute one quartette, and the four apical cells another, while, according to the usage here employed, all of the eight cells resulting from this cleavage would still belong to the same quartette. As a substitute for the word "quartette" in the latter sense, the word "tier" has been employed; thus the products of cleavage of the first generation of ectomeres would be called the upper and lower tiers of the first quartette.

The different quartettes in Conklin's scheme are designated by coefficients, and the genealogy of the cells of a quartette is indicated by exponents. The upper cell, or the right one when the cleavage is equatorial, is indicated by the smaller exponent;  $2a^1$ , for instance, indicates the upper cell in the  $a$  quadrant of the second quartette,  $2a^2$  the lower. The upper product of the cleavage of  $2a^1$  would be  $2a^{1.1}$ , while  $2a^{1.2}$  would represent the lower cell.

*The Eggs and Egg Masses.*

The egg masses of *Planorbis* are flattened and rather firm, and are usually found adhering to stones or aquatic plants. The eggs proper are found in relatively large capsules, which are imbedded in a jelly-like mass, outside of which is a somewhat tough enclosing membrane. The amount of jelly between the

capsules is quite small; in fact, the sides of the capsules are generally in contact, leaving only the interstices to be filled by this material. The amount of albumen in the capsules, in comparison with the size of the egg, is, on the other hand, very large. The diameter of the egg measures about .13 mm., while the diameter of the capsule is .6 mm. The egg mass is of a yellowish color in the species studied, and sufficiently transparent to enable one to successfully study the living embryo *in situ*. There is less jelly than in the egg masses of *Physa* and *Lymnaea*, and the membrane surrounding the capsule is less tough. The eggs may be readily teased out of the capsules, whereas in *Physa* and *Lymnaea* the capsules come out of the jelly entire, and slip around like rubber balls when the attempt is made to tease them apart by needles.

The time during which *P. trivolvis* lays its eggs extends from early spring until the fall. The eggs are deposited in the greatest abundance in the spring. Where the snails are abundant, stones may frequently be found almost entirely covered by the egg masses. Often the snails themselves have several clusters of eggs attached to different parts of the shell, and sometimes the egg masses may be found adhering to the bodies of aquatic insects. When kept in an aquarium, the snails, in the early part of their laying season, readily deposit their eggs on the glass; but later in the year, when the eggs are produced in less abundance, the animals become apparently more particular as to where they lay, and seldom deposit their eggs unless upon stones or aquatic plants, upon which they find minute algae, which serve for food.

Usually a capsule contains but one egg, but sometimes it may have two, and rarely three or more. When there are more than one egg in a capsule, often only one of them develops normally, yet two embryos in a late stage of development may sometimes be found in the same capsule. It is common to find some of the eggs in a cluster developing abnormally, and the proportion of such eggs is increased when the water becomes contaminated. If eggs are obtained from snails kept in an aquarium, care has therefore to be taken that the water is kept fresh and pure. With proper feeding and attention,

Planorbis can be made to lay eggs even in the winter months. Some of the snails I kept in glass dishes deposited eggs in the latter part of January, and the cleavage of these eggs was perfectly normal.

The unsegmented eggs of Planorbis are almost spherical in form and of a bright yellow color. The yolk, which gives the egg its yellow color, is somewhat more dense at the vegetal pole, although the difference is not strongly marked. The fresh egg, when seen through the microscope by transmitted light, shows a somewhat darker lower pole—the future entoderm—shading gradually into a lighter upper hemisphere—the future ectoderm. The opaque matter of the egg consists of two elements, small granules and larger globules of deutoplasm. The spheres of deutoplasm are found scattered through all portions of the egg with the exception of a very small, clear, protoplasmic area at the animal pole. The polar bodies are small and clear. The first one is the larger and of almost spherical form, and is carried on the top of the second polar globule. The polar bodies remain in connection with the egg until quite a late period of cleavage, when they drop off and disappear. The nucleus is situated in the small protoplasmic area at the animal pole.

#### *The First Cleavage.*

At the beginning of the first cleavage the clear protoplasmic area at the upper pole of the egg increases in size and elongates as the chromosomes are separated. The asters form dense radiating masses of fibers which are clearly visible in the living egg. Their general appearance is very similar to those of Physa, which are figured by Kostanecki ('96). The cleavage furrow appears first at the animal pole and gradually extends downward on either side, finally surrounding the egg. The constriction is deeper at the animal pole, as is the rule with yolk-laden eggs in which the spindle usually lies above the center. After the separation of the parts of the egg is complete, the daughter-cells become nearly spherical in form and come in contact at only a small portion of their surface.

The two cells are equal in size, as is the rule in gastropod eggs in which there is not a very large amount of yolk. The nuclei are large and vesicular, containing, in proportion to their size, only a small amount of chromatin. The yolk spheres again encroach upon the protoplasmic area around the nuclei, from which they had been extruded during mitosis, and the cells gradually flatten against each other until they resemble a single undivided sphere. The behavior of the yolk in relation to the processes of cell division seems to indicate that the regions around the poles of the spindle are the seat of a tension which squeezes out the deutoplasm spheres as the contraction of a sponge would squeeze out the water contained in its meshes. With the disappearance of the astral radiations and the decrease of tension in those regions, the yolk spheres become free to distribute themselves more uniformly through the egg. It seems probable that the rounding off of the cells after division is due to the persistence for a time of the same central tension which excluded the deutoplasm spheres from the region of nuclear division, and that the subsequent flattening of the cells after the resting period has begun is due to a relaxation of the tension which at the same time permits the more even distribution of the yolk, and allows those agencies tending to draw the cells together to become dominant. This flattening of the two blastomeres upon each other occurs very soon after their complete separation, and it continues until each blastomere becomes almost a hemisphere. A lenticular cleavage cavity, if we may call it such, makes its appearance at this stage, reaching its maximum development just before the next cleavage.

### *The Second Cleavage.*

The two cells usually begin to divide at the same time. The division of one cell sometimes occurs a short time before that of its fellow, but the cleavage is never completed before the process of division in the other cell is well under way. Both spindles are at first parallel to the plane where the cells join. When the elongation of the cells occurs, the spindles



turn slightly towards the right, and the cells themselves, as they lengthen, undergo a twisting around in the same direction. The first cleavage plane, when viewed from the animal pole, is bent first to the left and then to the right. The reason of this is that the cleavage is not perfectly horizontal, but two of the cells lie a little higher than the others. When the division is completed, there result four cells nearly equal in size, two of which, *B* and *D*, come in contact below, in the ventral cross furrow, while the other two, *A* and *C*, meet in a cross furrow at the upper pole, which is nearly at right angles to the lower one. The ventral cross furrow makes a negative angle of about  $45^{\circ}$  with the first cleavage plane, while the upper cross furrow makes with this plane an equal positive angle. The bending of the first cleavage furrow at the lower pole of the egg is the reverse of what occurs in *Crepidula* and other mollusks with dextrotropic cleavage. It is a rule, holding good for all known cases, that in dextrotropic cleavage the ventral cross furrow, when viewed from the animal pole, bends to the right, while in forms with reversed cleavage it bends to the left. The cross furrow at the animal pole of the egg, however, does not show such a constant relation to the first cleavage plane. In many cases it may be absent entirely, the four blastomeres meeting above in a point. In some cases it is parallel to the ventral cross furrow (*Crepidula*), in others it is nearly at right angles to it (*Nereis*, *Planorbis*, *Physa*, *Lymnaea*). These variations obviously depend upon the fact that in some cases the two cells *B* and *D* meet above as well as below,—in which case the two polar furrows would be parallel,—while in other eggs the alternate cells *A* and *C* meet at the animal pole and form a cross furrow at right angles to the lower one. It is not surprising, therefore, that the polar furrows should sometimes present different relations to each other in eggs of the same species.

The four cells round off after division like those produced by the previous cleavage. They become almost spherical, but they subsequently draw together and assume very nearly the form of a single undivided sphere. A cleavage cavity occurs between the two pairs of cells *AB* and *CD*; that is, between

those cells which were not separated by the last cleavage. In addition, a central cleavage cavity occurs somewhat later which reaches a considerable size and assumes a quadrilateral form. All of these cavities disappear during the next cleavage.

### *The Third Cleavage.*

The third cleavage in *Planorbis* is laeotropic. As in some other forms, the amount of rotation is more pronounced during the later stages of cell division, and the cells of the upper quartette finally lie in the angles between the larger lower cells. After the shifting has taken place the cells flatten, and there results once more an almost spherical mass of cells. A central cleavage cavity again makes its appearance, and again disappears during the next cleavage. The four upper cells resulting from this division, or the first quartette of ectomeres, are formed of clear granular protoplasm, which gives them an appearance quite distinct from the lower cells which contain the yolk. While much smaller than the lower cells, or macromeres, they are considerably larger than the corresponding cells in the eggs of most gasteropods. The cells *1a* and *1c* meet in a polar furrow, which is inclined at a considerable angle to the polar furrow at the vegetal pole.

It seems probable, as suggested by Lillie, that Rabl has made some errors in the orientation of the eggs figured in his first plate, which tend to produce confusion regarding the relation of the two polar furrows. In the first place, Rabl's figures indicate that, in the four-cell stage, the upper and lower cross furrows are parallel, which is the reverse of what occurs in the species here described. In case *B* and *D* meet above, as well as below, we might expect that their derivatives, *1b* and *1d*, would also meet in a cross furrow, whose angle with the lower furrow would approximately measure the amount of rotation of the micromeres. Kofoid's Fig. *D* ('95, p. 53) would represent their relation under such a supposition. Rabl's Fig. *11A* indicates a further rotation of the upper polar furrow until it lies at right angles to its original position. Fig. *12A*, on the other hand, shows this furrow at right angles to its position in *11A*.



There is doubtless an error in the orientation of this figure, if not in the figures of the cross furrow in some of the preceding stages.

In *P. trivolvis* the upper polar furrow would lie at right angles to the lower one, were it not that the shifting of the micromeres from right to left lessens this angle to one of about  $45^\circ$ . The eight-cell stage in *P. trivolvis* differs from that figured in Kofoid's Fig. *D* in that the upper polar furrow lies between *1a* and *1c* (or the cells which Kofoid has called  $b^{4,2}$  and  $d^{4,2}$ ) instead of *1b* and *1d* ( $a^{4,2}$ ,  $b^{4,2}$ , Kofoid), and is placed at right angles to the one there figured. The upper polar furrow makes a positive instead of a negative angle of  $45^\circ$  with the lower polar furrow taken as an axis.

*From the Eight to the Twenty-four Cell Stage.*

The four macromeres are the next cells to divide. This cleavage is dextrotropic, *i.e.*, in the reverse direction to the previous one. The second quartette of ectomeres thus formed is, like the first, composed of rather large clear cells, which, however, are markedly smaller than the macromeres. The cells flatten out after division, as after the preceding cleavages, so that the furrows between the cells almost disappear, and the cell outlines are marked only by narrow clear lines.

From the twelve-cell stage the egg passes quickly to that of twenty-four cells. The first quartette of ectomeres are the first cells to divide, the division occurring in a right-handed spiral. Very soon spindles occur almost simultaneously in the cells of the second quartette and in the macromeres. The cleavage of the cells of the second quartette is laetotropic, and the resulting cells are nearly equal in size. The division of the macromeres, which is likewise laetotropic, gives rise to the third generation of ectomeres. The cells of the third generation are large, and are marked off sharply from the macromeres, even before the division is completed, by their clear protoplasm. From their superficial outline they appear equal in size to the macromeres, or even larger. Their actual bulk, however, is less, as may be seen in optical sections, for they do not extend so far into the interior of the egg.

The twenty-four-cell stage, which is reached by these divisions, marks a resting stage of considerable length in the development of the egg. A cleavage cavity is formed at this period, which may attain quite a large size. In the arrangement of the cells the egg presents a perfect radial symmetry. The macromeres, or entomeres, as it is better now to call them, are somewhat larger than the cells above them, and their yellowish color and greater opacity, due to the yolk they contain, render them easily recognizable. Lying in the angles between the four entomeres are the cells of the third quartette. These cells are elongated in a meridional direction, the direction of their next cleavage. Alternating with these cells, and hence lying opposite the entomeres, are the lower cells of the second quartette,  $2a^2$ ,  $2b^2$ ,  $2c^2$ ,  $2d^2$ . The four entomeres are, therefore, surrounded by a circle of eight cells, of the second and third quartettes. The four upper cells of the second quartette, when viewed from the upper pole, lie a little to the left of the lower ones, and do not come into contact with the cells of the entoderm. At the upper pole of the egg there are eight cells, the products of the cleavage of the first quartette of ectomeres; the four lower cells, the trochoblasts, alternate with the cells of the second quartette, and lie opposite those of the third with which they are in contact. The relation between these cells corresponds essentially to the relation between the groups of cells arising from them, even in a late stage of development. The descendants of the four upper cells go entirely into the formation of the cross, which will be described later; below these cells lie those of the second quartette, and below these again the entomeres. Alternating with these groups, there occur, in vertical arrangement, the trochoblasts, the third quartette, and the angles between the entomeres. This arrangement of the various quartettes, which is typical for molluscan spiral cleavage, is a great aid in following their further history.

There are a few points in which the cleavage of *P. trivolvis* differs from Rabl's account of the cleavage of the form studied by him, which it may be well to point out. Rabl's different account of the cross furrows has already been discussed. The passage from the twelve to the twenty-four cell stage takes

place, according to Rabl, by the simultaneous division of all of the cells of the egg. In *P. trivolvis* the first quartette divides before the others, forming a sixteen-cell stage. Finally, although Rabl says nothing concerning the direction of the cleavage of the cells of the second quartette, his Figs. 12*A* and 12*B* indicate that the division was dexiotropic. In *P. trivolvis* this cleavage is plainly laeotropic, and the cells lie much more nearly in a vertical plane than they are represented in Rabl's figure. There can be no doubt that the first quartette in the forms studied by Rabl is given off in a laeotropic direction. He expressly states this, and it is also indicated by his figures. The second quartette, according to the law of alternation of spirals, should be given off in a dexiotropic direction. If, as Rabl's figures indicate, the second quartette divides dexiotropically, there would result two dexiotropic divisions in immediate succession. It seems more probable that Rabl's figures are misleading on this point than that there should be an exception to the law of alternation of spirals at this early period of cleavage. It is not unnatural that, not having in mind any significance attached to this point, an observer should be in error regarding it.

The cleavage of the eggs of *Physa* and *Lymnaea* as far as the twenty-four-cell stage is essentially the same in almost every point as that of *Planorbis*, and there is a remarkable agreement between the cleavage of *Planorbis* and *Limax*, as described by Kofoid and Meisenheimer, as far as the cell lineage in the latter forms was carried. The cleavage of the eggs of the pulmonates, so far as they have been studied, seems, in fact, to be characterized by several points of marked similarity. The amount of yolk in the eggs is not great; the ectomeres are large; there is a recurrent cleavage cavity; and vacuoles are often formed between the blastomeres. In the twenty-four-cell stage in the above forms the cells have essentially the same relative size and arrangement, and the entomeres are scarcely larger than the other cells of the egg. The eggs of pulmonates complete their development in capsules which contain a very large amount of fluid albuminous substance, which serves to nourish the embryo. As the amount of food in the form of albumen is large, it is

natural that the amount of food in the form of yolk should be small, and the more nearly equal size of the blastomeres in the early cleavage stages of pulmonates, in comparison with the eggs of most marine forms, is probably due to the relatively small amount of yolk in the egg. It is the rule among gastropods that the greater the amount of yolk in the egg the smaller are the ectomeres in relation to the entomeres. A comparison of such yolk-laden eggs as those of *Purpura* and *Nassa* with the eggs of *Crepidula* and *Umbrella*, or these again with the eggs of *Paludina* or the pulmonates, will illustrate this principle very forcibly. It has been pointed out by Kofoed, as a rule holding for a great variety of forms, that "the greater the amount of yolk, the greater seems to be the tendency of the cells of a yolk-laden quartette to divide before those of the smaller quartette." For instance, in the eggs of *Limax*, which have little yolk, both macromeres and ectomeres at the eight-cell stage divide almost simultaneously, and the egg passes at once to the sixteen-cell stage. In the eggs of *Planorbis*, *Physa*, and *Lymnaea*, which contain somewhat more yolk, the macromeres divide before the ectomeres, giving rise to a stage with twelve cells. In the eggs of *Umbrella* and *Urosalpinx*, which contain a still larger amount of yolk, even the third quartette is given off before the first has divided. These facts can scarcely be said to show, however, that the presence of yolk in cells actually accelerates their division, as Kofoed seems to imply. It is a general rule that the larger a cell is, the sooner it tends to divide. There are numerous exceptions to this rule, some of which will be pointed out later, but it expresses a more or less dominant tendency in the cleavage of the egg. A large amount of yolk in the egg, moreover, would determine the micromeres to be of small size, and the small size of these cells would tend to delay their cleavage. The yolk may, and probably does, delay the cleavage of the cells containing it, but the small size of the ectomeres in yolk-laden eggs delays their cleavage even more. It is probably for this reason that we find a delayed cleavage of the ectomeres in yolk-laden eggs. And this conclusion is in harmony with the fact that the cleavage in yolk-laden eggs is usually slow.



As, perhaps, in all other mollusks, except the cephalopods, and in the annelid worms, all of the ectoderm is contained in the first three quartettes of micromeres. The three macromeres, *A*, *B*, and *C*, are entirely entodermic, while the posterior one, *D*, contains both entoderm and mesoderm. The cases in which more than three quartettes of ectomeres are said to be formed I think we must regard, with Conklin, as open to serious question. In the case of *Fulgur*, in which a large number of quartettes of ectomeres was said to be formed, Conklin has shown that there is, in reality, only the usual number, three. In *Nassa*, Bobretzky held that the macromeres budded off a large number of ectomeres; but Conklin finds that in the closely related genus, *Ilyanassa*, the usual number of quartettes is produced. Salensky has asserted that more than three quartettes of ectomeres are formed in *Vermetus*, and Erlanger has made the same statement regarding *Bythinia*. But in *Serpulorbis squamata*, from the coast of California, — a form very closely allied to *Vermetus*, in which genus it was, in fact, originally placed, — I have found that the whole ectoderm arises from but three quartettes.

It is not so much the fact that the cases reported of the formation of more than three quartettes of ectomeres are exceptions to a general rule that makes them so improbable — it is the very definite and similar fate of these quartettes in all the forms in which their history has been traced. Speaking of this rule that the ectoderm in annelids and mollusks arises from three quartettes, Dr. Conklin says, "The cause of this remarkable phenomenon is to be found in the fact, I believe, that each of these quartettes of ectomeres is the protoblast of definite regions of the embryo." Each quartette forms essentially the same parts of the embryo in every mollusk that has been studied in this regard. An additional quartette would necessitate a considerable modification of the fates of the preceding quartettes. Were the fates of the different cell generations to a large extent indeterminate, a variation in their number would not seem so great an improbability. Since, however, each quartette has essentially the same destiny, not only in widely separated groups of the Mollusca but also in annelids, we have very strong reasons,

I believe, for holding the accounts of the formation of four or more generations of ectomeres to be erroneous.

The last author who records more than three generations of ectomeres is Fujita, who studied the cleavage of the pulmonate *Siphonaria*. After describing the formation of the four-cell stage, he says: "During the next following stages four successive generations of micromeres are budded off from each of the above-mentioned segments, now to be called macromeres. Hereupon the macromere *D* is entitled to the name of entomesoderm, and the remaining three macromeres may be called entodermic macromeres. Synchronously with the formation of the fourth generation of micromeres, each member of the third generation divides, thus giving rise to a fifth generation. At this stage there are twenty micromeres and four macromeres, the relations of which may be seen in Fig. 2. Next comes in order the formation of a sixth generation of micromeres again from the third, followed by that of a seventh from the fifth." It seems to me quite certain that Fujita overlooked the division of the first generation of micromeres and concluded that the four outer cells resulting from this cleavage arose from the macromeres. This is a very natural and easy error to make, as I can testify from experience, having been deceived, for a time, on just this point, when working on another form. Fujita describes no division of the first quartette until after the stage in which the egg contains thirty-four cells, when the cells of the second quartette have divided twice, and the posterior cells of the third have divided. Moreover, Fujita's figure of the twenty-four-cell stage shows that it corresponds exactly, so far as the relations of the cells are concerned, with the same stage in *Planorbis*, *Physa*, and *Crepidula*. If we assume that the cells marked 2 in Fujita's Fig. 3 are the trochoblasts, the genealogy of all the cells would exactly correspond to that in the above forms, and the following divisions up to the forty-third-cell stage, which Fujita describes, would correspond point for point with those of *Planorbis*. The cells 2 are smaller than the apical cells, and their position indicates that they arose by a laeotropic division, as would be expected according to the principle of alternation of spirals. All of these



facts, taken in connection with the antecedent improbability of Fujita's conclusion, make it appear very probable that the egg of *Siphonaria* contains but the usual number of quartettes of ectomeres.

*Cleavage Cavity and Vacuoles.*

The existence of a recurrent cleavage cavity seems to characterize, in an especial manner, the cleavage of the pulmonate gasteropods. It has been observed by several workers on these forms (Warneck, Fol, Rabl, Brooks, Schmidt, Meisenheimer), and has been described so fully in the case of *Limax* by Kofoid, that it will only be briefly considered here. In *Planorbis* the cleavage cavity does not attain nearly such extensive development as in *Limax*. Whether this relation obtains between the aquatic and terrestrial pulmonates in general is uncertain, though in the aquatic forms, *Physa*, *Lymnaea*, and *Planorbis*, the cleavage cavity is not so large as in the terrestrial genera, *Limax* and *Succinea*. In *Planorbis*, as in all the above forms, the cleavage cavity first appears in the two-cell stage. This disappears at the next division; and, in the four-cell stage, two cavities appear between the two pairs of cells *AB* and *CD*, and, in addition, there develops a central cleavage cavity which assumes a quadrate form and finally merges with the other two. A similar occurrence repeats itself at the eight-cell stage. With each cleavage the cavity disappears, then forms again, and gradually increases in size until the rounding off of the cells during the next cleavage causes a break in the wall and allows the fluid contents to escape. From the twenty-four-cell stage on, the cleavage cavity appears to be permanent.

Small intercellular vacuoles occur in a late period of cleavage, as in *Limax*, and they are found most abundantly in the ectodermic portions of the egg. All of these cavities, as pointed out by Kofoid, seem to be the result of excretory activity. (For a discussion of the function and occurrence of the cleavage cavity in different forms, see Kofoid ('95), p. 81.)

*From the Twenty-four to the Forty-nine Cell Stage.*

*The Division of the Second Quartette and the Formation of the Cross.*—The transition from the twenty-four to the forty-nine cell stage occurs very quickly. The upper tier of the second quartette divides in a dextrotropic direction. The upper cell, as in *Crepidula*, *Umbrella*, and *Unio*, is the smaller, and Meisenheimer's Fig. 31 shows the same is true also in *Limax*. These upper cells, as in the above forms, form the tips of the arms of the cross presently to be described. Blochmann was doubtless wrong in his derivation of these tip cells in *Neritina*, and I think Conklin's correction of this mistake is to be followed, rather than that given by Kofoed, as it brings the cleavage of *Neritina* into complete harmony with that of other mollusks (see Conklin ('97), p. 64). The tip cells of the lateral arms of the cross in *Neritina* were found by Blochmann to have a peculiar granular appearance, and were held by him to give rise to the velum. It is probable, however, that only a portion of the velum is formed from these cells, as in both *Crepidula* and *Planorbis*, and also in *Ischnochiton*, certain cells of the first quartette, and other cells from the second also, go into the formation of this organ. The granular character of these tip cells in *Neritina* has not, I believe, been seen in any other form.

The division of the upper tier of the second quartette is soon followed by that of the lower. This cleavage is also dextrotropic, but the smaller cell is now the lower one. The upper cell, resulting from this division, lies at the side of the lower cell, arising from the previous division. There are now four groups of four cells each, or sixteen cells, in the second quartette. In each group there is a pair of large cells situated side by side, and a smaller cell above and one below. The lower cells lie opposite the entomeres.

The apical cells of the first quartette now divide in a laeotropic direction. When this division is completed, the arrangement of certain cells of the upper pole becomes such as to give the appearance of a cross. The outer cells of the first quartette,  $1a^{1,2}$ ,  $1b^{1,2}$ , etc., form the bases of the arms of the cross,











the inner ones forming the center, and the cells of the second quartette forming the tips. At its first appearance the cross, therefore, contains twelve cells, eight belonging to the first, and four to the second quartette. From this stage until the period of gastrulation, the cross is a very conspicuous feature of the egg. Its further history will be traced in a later section.

*The Trochoblasts.*—The cleavage of the apical cells of the egg is soon followed by a division of the four outer cells of the first quartette, the trochoblasts,  $1a^2$ ,  $1b^2$ , etc. This division is nearly radial, the upper cell lying in the angles between the arms of the cross directly above the lower one. The two cells are about equal in size and they soon begin to enlarge and become clear. One peculiarity of this cleavage is that it occurs at a much earlier stage than in *Umbrella* and *Crepidula*. In the first genus the trochoblasts do not divide until the egg contains more than seventy cells; in the second the anterior trochoblasts do not divide until the number of cells in the egg is over one hundred, while the posterior trochoblasts do not divide until later, if at all. In *Unio*, however, they divide at about the fifty-cell stage, and at a still earlier stage in *Chiton* (Metcalf) and *Ischnochiton* (Heath). If we compare the egg of *Planorbis* with that of *Limax* in this respect, we find a close agreement. The time at which this division takes place in *Limax* is, according to Kofoed, quite variable, but it occurs, speaking roughly, at about the forty-cell stage. The cells in *Limax* are comparatively large, as in *Planorbis*, but, strangely enough, they divide in an entirely different direction. "The axis of the spindle," says Kofoed, "lies parallel to the plane of the equator. There is every indication that the division is nearly meridional (horizontal)." Meisenheimer's Fig. 31 shows horizontal spindles in all four trochoblasts in the egg of *Limax maximus*. The cells resulting from this division lie nearly side by side, instead of the one above the other, as in *Planorbis*, there being only a slight dextrotropic tendency in the cleavage (see Kofoed, '95, p. 59, Fig. 41). In Conklin's Fig. 50 the cells of the two anterior pairs of trochoblasts lie in nearly the same horizontal plane, and their symmetrical position in relation to the anterior arm of the cross indicates that they were produced

by a bilateral division. At a late period the anterior trochoblasts in *Planorbis* become so shifted as to lie in very nearly the same position as in *Crepidula*. It is probable that there is no other group of cells which presents, in different mollusks, such a remarkable degree of variation, both in the time and in the direction of their cleavage. Yet they have essentially the same fate not only in mollusks, but also in annelids. There is, I believe, no reasonable escape from the conclusion expressed by Conklin, that the trochoblasts in annelids and mollusks are truly homologous. Having the same origin, position, and fate in both these groups, the evidence of their homology is as complete as ontogeny can furnish.

In most gasteropods the trochoblasts are of small size. This is especially the case in *Neritina*, *Umbrella*, and *Crepidula*, and, according to Conklin, they are small also in *Urosalpinx* and *Fulgur*. In the pulmonates, however, their size is larger. In the preceding cases the cells of the first quartette are very small in relation to the macromeres and they divide unequally, the outer cells being much smaller than the apical ones. In *Limax agrestis* (Kofoid) and in *Planorbis* the cells of the first quartette are not only much larger than in the above forms, but they divide into almost equal parts. In the twenty-four-cell stage of both forms the trochoblasts have about the same size as the other cells of the egg. Their bulk is, therefore, vastly greater than that of the corresponding cells in *Umbrella* or *Crepidula*. Has not the relatively large size of the trochoblasts in *Limax* and *Planorbis* some causal connection with their precocious divisions? Is not their size dependent largely on the size of the cells of the first quartette, and this, again, upon the relatively small amount of yolk in the egg? It seems probable that the amount of yolk in the egg may indirectly influence the size of the trochoblasts and the time at which they divide. It is not contended that this is the only factor in the case. It does not explain, for instance, why the cells of the first quartette divide almost equally in some cases and very unequally in others. But when we compare the eggs of *Unio*, *Planorbis*, and *Limax* with those of *Umbrella* and *Crepidula*, it is difficult to resist the impression that yolk is, in great measure, respon-

sible for the great difference in the period at which the division of the trochoblasts occurs.

It might be urged that if the large size of the trochoblasts were the cause of their early division, in forms with a relatively small amount of yolk, they ought to continue to divide, since they grow more rapidly than perhaps any other cells of the egg. In *Crepidula* these cells are at first "much the smallest cells of the entire egg"; finally they become, with the exception of the yolk cells, the largest cells of the egg; and their greater relative size is due not only to the fact that the other cells are getting smaller as division proceeds, but their absolute bulk is greatly increased. Yet neither in *Crepidula* nor in any other gasteropod has more than one cleavage of these cells been observed, while in *Ischnochiton*, according to Heath ('99), and in *Amphitrite*, *Lepidonotus*, and *Clymenella* among annelids, according to Mead, they stop dividing entirely after the second cleavage. The cessation of the cleavage of these cells is doubtless connected, as Conklin maintains, with their prospective destiny. They soon acquire cilia and become a part of a specialized organ. This necessitates a special modification of their structure, which, setting in at an early period, checks the tendency to division. It is well known that when the differentiation of a cell is carried very far it generally ceases to divide, or if it does divide, its specialized structure in great part disappears and it returns to a more embryonic condition. And we probably have in the cessation of the division of the trochoblasts simply an exhibition of this rule. The trochoblasts in *Planorbis*, after they have divided, become much flattened or thinned out. In eggs stained with silver nitrate they are almost transparent, while the cells of the cross are stained brown, causing this structure to appear in conspicuous contrast to the cells between the arms. With the growth of the trochoblasts the arms of the cross become more narrow, as if they were pressed together at the sides. They thus take a somewhat darker stain and stand out in a more conspicuous manner than before. The further history of the trochoblasts will be described in the section on the cell lineage of the prototroch.

Although only the anterior pair of trochoblasts in *Planorbis* enters into the formation of the prototroch, the term "trochoblasts" has been applied to the posterior pair as well. The latter form a portion of the head vesicle. As this structure may be considered as consisting mainly of the enlarged posterior portion of the prototroch, it would scarcely be incorrect to call these cells trochoblasts also.

*The Division of the Third Quartette.*—The cells of the third quartette are large and elongated in a meridional direction. Their cleavage is almost exactly radial, as in *Limax*. In *Crepidula*, according to Conklin, "the direction of the cleavage is nearly radial, though after the cleavage has occurred it is seen to be plainly laeotropic in  $3a$ ,  $3b$ , and  $3c$ , and dextro-tropic in  $3d$ , *i.e.*, the cleavage is nearly bilateral on the posterior end of the ovum." Yet the spindle in  $3d$  is sometimes laeotropic, as Conklin adds, and the nuclei may show this relation to each other, while "the cell body may show reversal of cleavage." "This," to quote the same author again, "is but another illustration of the fact that bilaterality first appears on the posterior side of the egg, that it is due to the change in direction of one out of four cells, and that it is not perfect when it first appears, but is merely a deviation from the spiral type toward the bilateral." In *Planorbis* the two cells on the posterior side of the egg,  $3a$  and  $3d$ , divide before the anterior ones,  $3b$  and  $3c$ , but I have been unable to find any constant deviation from the radial direction of their cleavage. A radial cleavage may, however, be considered an approach toward the bilateral type, and we may view the earlier division of the cells  $3a$  and  $3d$  as the first foreshadowing of bilateral cleavage.

The lower cells of the third quartette are somewhat smaller than the upper ones and have their long axis horizontal, while the long axis of the upper cells is still radial. In *Neritina* and *Umbrella* the cleavage of the cells of this quartette is also nearly radial, and the lower cell is the smaller, as is also the case in *Crepidula*, and, according to Kofoid's and Meisenheimer's figures, in *Limax*. In *Physa*, according to Wierzejski, the cleavage is also meridional and unequal, but in the anterior



quadrants the smaller cell is nearer the animal pole, while on the posterior side of the egg the reverse is the case. Which of the cells are the first to divide is not stated. In another pulmonate, Siphonaria, the division of the posterior cells of the third quartette occurs before that of the anterior ones, each cell giving off a small cell toward the vegetal pole. The division in both cells is laeotropic, but less so in the cell on the left side (Fujita, '95, p. 91, Fig. 5, 6x). This cleavage, according to Fujita, occurs at the thirty-two-cell stage; the corresponding cells in the anterior quadrant have not divided at the stage in which the egg contains forty-three cells, which is as far as the cell lineage of this form is described.

The first division of the cells of the third quartette seems to mark the point in the cleavage of gasteropods where bilateral cleavage makes its first uncertain appearance. In no case is there a typical spiral cleavage of all the four cells. The cleavage may be radial (Physa, Limax, Planorbis, Neritina, Umbrella), slightly bilateral in the posterior quadrants, and spiral in the anterior ones (Crepidula), or spiral in the posterior quadrants, but with an approach toward the bilateral type (Siphonaria). The posterior quadrants generally divide before the anterior ones. In Limax, however, the order of cleavage in the different quadrants seems to be inconstant (Kofoid). In Crepidula, Umbrella, and Planorbis the posterior cells divide only a short time before the anterior ones, while there is a long interval between these divisions in Siphonaria. The cleavage of the cells of this quartette is, in all the above forms, unequal, and, except in the anterior quadrants in Physa, the smaller cell lies nearer the vegetal pole of the egg.

*The Formation of the Primary Mesoblasts.* — Soon after the twenty-four-cell stage is reached, the posterior macromere *D* divides into unequal parts. The upper moiety is much larger than the lower one, and, from the first, lies partly pushed into the cleavage cavity, so that only a small portion of it appears at the surface of the egg (Pl. XVII, Fig. 11). It is dark and granular, and contains a large amount of yolk, like the cells of the entoderm. The division of *D* is dextrotropic, as Crampton found it to be in Physa. The primary mesomere lies, therefore,

to the left of  $D$  in forms with reversed cleavage, and to the right of  $D$  in forms in which the cleavage is not reversed. Before the division of  $D$ , the two macromeres,  $B$  and  $D$ , were equal in size, and the anterior and posterior sides of the egg could not be distinguished. The position of  $4d$  or  $M$  enables one henceforth to easily locate the posterior side of the egg. The primary mesomere soon divides in a nearly horizontal direction, though the cleavage is slightly oblique. This division is completed, as in *Siphonaria*, before the other cells of the fourth quartette arise. The two mesomeres gradually sink further into the egg and lose connection with the surface at about the sixty-four-cell stage.

One cannot compare the formation of the primary mesoblast in the different pulmonates, in which its origin has been traced, without being struck with the very close similarity of the process in the several forms. The exact cell origin of the primary mesoblast has been determined in *Limax*, *Physa*, *Lymnaea*, *Planorbis*, and *Siphonaria*. In all these forms the mesomere arises, shortly after the twenty-four-cell stage is reached, by the oblique division of  $D$ ; it lies, from the first, partly pushed into the cleavage cavity; it is much larger than the entomere  $D$ , and of an opaque appearance; and it soon divides in a nearly horizontal direction and gives rise to the mesoblastic bands. A similar origin of the primary mesoderm is found in several other gastropods, but, with the exception of *Umbrella*, the mesomere is considerably smaller than the entoderm cell  $D$  (*Bythinia*, *Crepidula*, *Neritina*, *Ilyanassa*, *Fulgur*). There are at present many divergent accounts of the origin of the mesoblast in the mollusca. These accounts have recently been reviewed by different writers on the subject (Heymons, '93, Tönniges, '96, Schmidt, '95, Meisenheimer, '96), and fully discussed in Korschelt and Heider's *Embryology*, so that it would be superfluous to devote space to the subject here. In almost every case, however, in which the cleavage has been followed with sufficient care, the primary mesoderm has been found to arise, as in the above forms, from the posterior macromere  $D$ . Yet careful study has failed to discover pole cells in *Paludina vivipera*, but the fate of the cell  $4d$  in this form has never been traced.



What becomes of this cell in this form would be a matter of considerable interest.

*The Fourth Quartette.*—The fourth quartette is produced by a dextrotropic cleavage of the entomeres. All the cells of this quartette are large and full of yolk; the four cells at the vegetal pole are small and clearer than the others. They are quite thin and, therefore, less in bulk than their superficial area would indicate. The general appearance of the entoderm cells at this stage is very similar to those of *Limax*, and markedly different from those of most marine forms, in which there is usually much yolk.

The number of cells in the egg when the fourth quartette is formed is forty-nine.

Cells of the first quartette . . . . .	16	{ 8 trochoblasts 8 apical cells
Cells of the second quartette . . . . .	16	
Cells of the third quartette . . . . .	8	
Cells of the fourth quartette . . . . .	5	{ 2 mesomeres 3 entomeres
Cells at the vegetal pole . . . . .	4	

One of the most conspicuous features of the egg at this stage is a belt of large cells around the equator. It is composed of twelve cells, the four middle pairs of cells of the second quartette alternating with the four upper cells of the third. All of these cells are more or less oblong, with their long axes vertical. A similar belt of cells may be seen, though less plainly, in *Crepidula* and *Limax*.

*The History of the Cross.*

At the time of its first appearance the cross contains eight cells of the first and four cells of the second quartette. Its center is at the apical pole of the egg, and its arms are anterior, posterior, right, and left. The tips of the cross lie over the large entomeres of the fourth quartette, the cells of the second quartette lying between. The median plane of the egg would cut through the middle of the anterior and posterior arms, and the entomere 4*b*, and pass between the two mesoblasts. Previous to the formation of the fourth quartette, this plane would

have cut the ventral cross furrow at almost a right angle. After the fourth quartette is formed, owing to the shifting of the small cells at the vegetal pole, the cross furrow would be cut at an oblique angle. Viewed from the animal pole, the cross furrow has been turned several degrees in a laeotropic direction. This rotation is obviously a result of the dextrotropic cleavage of the macromeres. Of course, if we take the small cells at the vegetative pole as fixed, we might consider that the rest of the egg, the fourth quartette and the ectomeres, had rotated in an opposite, or dextrotropic, direction. In an egg like that of *Crepidula*, in which the homologues of these small cells form the greater part of the bulk of the egg, it is natural to regard the furrows between these cells as fixed, and to speak of a laeotropic rotation of the ectoblast rather than a dextrotropic rotation of *A*, *B*, *C*, and *D*. Whichever pole of the egg we regard as fixed,—and this is the essential point,—it is certain that the rotation in the two forms has taken place, in accordance with their reverse types of cleavage, in opposite directions.

The next cleavage in the cross occurs in the basal cells at about the sixty-four-cell stage. This division is nearly radial, though slightly laeotropic. It is an interesting fact that we have here a violation of the rule of alternation of spirals exactly where it first occurs in *Crepidula* and *Neritina*, but with this difference, that in *Planorbis* the two successive cleavages are laeotropic, while in *Crepidula* and *Neritina* they are dextrotropic.

The apical cells  $1a^{1,1}$ ,  $1b^{1,1}$ , etc., next divide. There seems to be a tendency to a dextrotropic cleavage in these cells, but the direction of the division appears to be more or less inconstant. To the extent that this cleavage may be considered dextrotropic, it is in accordance with the rule of alternation of spirals. This division marks the close of the period of definite spiral cleavage in the cells of the cross. The subsequent cleavages, to which this may be considered a transition, are all of the bilateral type. With the completion of this division the number of cells in the egg reaches 104. In *Crepidula* this cleavage is laeotropic, and Heymons mentions the fact that in *Umbrella* these cells divide, but says nothing concerning the direction of the cleavage. The











next cleavage occurs in the basal cells of the arms of the cross, and takes place in a radial direction. Each arm of the cross now contains three cells, besides the tip cells, which belong to the second quartette. This division is in the same direction as the preceding division of the basal cells, so there occurs a second exception to the rule of alternation of spirals. It is a noteworthy fact, also, that the direction of this division is at right angles to the corresponding cleavage in *Crepidula*. In *Crepidula*, when there are three cells in each arm of the cross, the two cells behind the small tip cells are much elongated transversely to the long axis of the arms, and it is natural that their cleavage should be at right angles to their longest diameter. The middle cells of the arms in *Crepidula* divide first; this division is followed by the cleavage of the cells at the apical pole of the egg, and soon afterward the division of the basal cells takes place. In *Planorbis* it is the apical cells that first divide; then the basal cells divide radially, so that before the longitudinal splitting of the arms occurs each arm has one more cell than in *Crepidula*; a radial division, lengthening the arms in *Planorbis*, takes the place of a transverse division, splitting the arms in *Crepidula*. Moreover, the cells lying just behind the tip cells of the cross, which in *Crepidula* divide first, in *Planorbis*, except in the anterior arm of the cross, *never divide again*.

The inner median cell of the anterior arm of the cross next divides transversely and marks the beginning of the splitting of the arms of the cross. After a short interval the splitting of the lateral arms also begins; the cleavages are slightly oblique, the lines of division pointing toward the anterior end of the cross. This nearly transverse division occurs only in the basal cells and the ones lying next to them, the inner median cells. *The basal cell in the anterior arm undergoes no further divisions*. Its history will be described later. The transverse splitting in the anterior arm at this stage is, therefore, limited to a single cell. The posterior arm of the cross, as in *Crepidula*, remains undivided throughout its entire history. With the longitudinal division of the basal cell above described, the history of the cleavages in the posterior arm is completed.

The composition of the cross at this period is as follows (Pl. XX, Fig. 37): Four rather small cells in the center,  $1a^{1.1.1}$ ,  $1b^{1.1.1}$ , etc., around the apical pole of the egg; around these are four other cells, the intermediate cells,  $1a^{1.1.2}$ , etc., in the angles between the arms; the anterior arm, which is broader than the others, consisting of a single basal cell,  $1b^{1.2.1.1}$ , then a pair of cells,  $1b^{1.2.1.2.1}$ ,  $1b^{1.2.1.2.2}$ , resulting from the transverse division of the inner median cell; then a single outer median cell,  $1b^{1.2.2}$ , and finally the tip cell,  $2b^{1.1}$ ; the two lateral arms consisting of two pairs of cells at the base, an undivided outer median cell, and a tip cell; the posterior arm, consisting of a row of four cells. In all, the number of cells in the cross is twenty-nine. The anterior arm is shorter than the others, and the tip cell is more or less clear. The arms of the cross are slightly oblique; and it is worthy of note that the direction of their inclination is laeotropic, while in *Crepidula* and *Ischnochiton* the arms show a slight dextrotropic twist. This difference is doubtless connected with reverse types of cleavage of these forms.

The tip cells of the arms have enlarged and become transparent. The tip cell of the right arm is widest behind, while that of the left is widest in front; the outer median cells show the reverse relation. The cells of the posterior arm of the cross are usually rhomboidal in outline. The posterior tip cell is large and elongated in the direction of the arm, while the long axis of the other tip cells is transverse to the arm. The anterior tip cell is the smallest of the four. As the tip cells in eggs stained with silver nitrate are clear, the portion of the cross which stains dark is that derived entirely from the first quartette. This portion is very conspicuous, as it is surrounded on all sides by transparent cells.

It may be well, before tracing the history of the cross further, to compare it briefly with the cross in other mollusks. Blochmann traces the history of the cross in *Neritina* to a stage in which each arm contained three cells, except the posterior arm, which was composed of four. Blochmann's probably incorrect derivation of the tip cells has already been mentioned, and Kofoed has shown also that it is almost certain that his deri-

vation of the basal cells of the cross was likewise incorrect. Interpreting Blochmann's figures in the light of what is known to be the rule in other forms, it is evident that the cell lineage of the cross in *Neritina*, at the stage when there are three cells in each arm, corresponds exactly to that of *Umbrella*, *Crepidula*, and *Planorbis*. In the lengthening of the posterior arm of the cross by the addition of another cell, there is a further point of agreement with the history of the cross in the last two forms. In *Crepidula* both the basal and the tip cells of the posterior arm of the cross divide radially at about the same time, so that this arm comes to have one cell more, instead of one less than the other three. Two of the four cells in this arm, therefore, belong to the first quartette and two to the second. Whether three of the four cells of the posterior arm in *Neritina* belong to the first quartette, or whether there are two cells each of the first and second quartette, is uncertain. Blochmann says nothing about the derivation of this additional cell. Should we argue from analogy with *Crepidula*, we should be led to accept Conklin's scheme of the probable derivation of this cell, and derive the two posterior cells from the second quartette. If, on the other hand, we should draw our conclusion from a comparison with *Planorbis*, we should infer that the three anterior cells belonged to the first quartette, while only the tip cell belonged to the second. In *Planorbis* the four cells in each arm have the same derivation and are produced at nearly the same time. In both *Neritina* and *Crepidula*, owing to a delay in the division of the basal cell, the posterior arm contains at first but two cells, while each of the other arms contains three; this stage is followed by a stage in which the posterior arm contains four cells, while the number in the other arms remains the same. The tip cell in the posterior arm in *Crepidula* is larger than those of the other arms and divides first, and in a different direction from the others; that is, radially instead of transversely. The divisions of the cross cells in *Planorbis* are such that they preserve the radial symmetry of the cross much longer than in *Crepidula* and *Neritina*. There is little difference, either in the time or direction of the cleavages, in the different arms until after the period in which each arm is composed

of over four cells. The radial symmetry of the cross is not destroyed until the egg contains over one hundred blastomeres. In *Neritina* the cross becomes a bilaterally symmetrical structure, by the lengthening of the posterior arm, when the egg contains only about fifty cells. In *Crepidula* the cross may be said to be bilaterally symmetrical from the very beginning of its formation, owing to the smaller size of the basal cell and the larger size of the tip cell in the posterior arm. At about the forty-eight-cell stage there are only two cells in the posterior arm and three in the others; the divisions which increase the number of cells in the posterior arm to four occur when the egg contains sixty-seven cells, at the same time that the first transverse division in the other arms is taking place. The appearance of bilateral symmetry in the cross in *Umbrella*, Heymons did not describe.

The striking differences in the history of the cross in *Planorbis* and *Crepidula* are most interesting. It is natural to seek for some explanations of the problems which these differences present to us. Why is it that the cleavage of the cell, which in *Crepidula* results in a splitting of the arm of the cross, produces in *Planorbis* a lengthening of the arm of the cross? Why does the posterior tip cell in *Crepidula* divide before the others, and in a different direction, while it does not divide at all in *Planorbis*? Why is the radial symmetry of the cross retained longer in *Planorbis* than in *Crepidula*? And what is the cause of the very different behavior of all of the tip cells in the two forms? These are a few questions which suggest themselves when we compare the history of the cross in these forms. A complete solution of these problems is at present impossible, but we may, perhaps, determine some of the proximate causes of these differences of behavior. The transverse division of the cells,  $1a^{1,2,1}$ , etc., in *Crepidula* is doubtless the result of the fact that the long axes of these cells are transverse to the arm of the cross. In *Planorbis* the basal cells in the arm, at the time they begin to divide, have their longitudinal axes in the contrary direction. Hence it is natural that their division should be radial and not transverse. Now the different shape of these cells in *Planorbis* is apparently due to the growth of the trochoblasts, which, as they increase in size, crowd the adjacent cells













together. Thus the arms of the cross would be subjected to a lateral pressure, which would tend to give the cells an elongation in a radial direction. In fact, as the trochoblasts increase in size, the arms of the cross actually do become narrower, as may readily be seen by comparing the figures of the earlier and later stages in the history of this structure. In *Crepidula* the trochoblasts are relatively much smaller than in *Planorbis*, and, at the time that the divisions above described occur, have not attained a sufficient size to exert much influence upon the arms of the cross. Thus the proximate cause of the difference in the direction of the division of the basal cells of the cross in the two forms seems very probably to lie in the different character of the trochoblasts. The cause of these differences in the trochoblasts may, as has been suggested above, lie partly, at least, in the relative size of the first quartette of ectomeres in the two forms, which, in turn, is largely dependent on the amount of yolk in the egg.

Are there any facts which throw any light on the different behavior of the cells in the posterior arm of the cross in the two forms? It will be remembered that in both *Crepidula* and *Planorbis* the posterior arm always remains undivided; *i.e.*, it consists of but a single row of cells. In *Crepidula* the development of this arm of the cross lags behind the others, the first basal cell not dividing until a considerable time after the others. Why is this? The explanation appears to lie in the fact that the cell  $1d^{1,2}$  is smaller than the other three basals. Conklin, I believe, does not mention the fact, but in all of his figures this cell is uniformly represented as smaller than the others (Figs. 23, 25, 26, 29-31), in one case (Fig. 31) the difference being very marked. Further back than this it is to be noted that the division of the cell from which the basal cell  $1d^{1,2}$  arose seems to be delayed, a fact which would indicate the smaller size of this cell, although this is not otherwise noticeable. It is certain, however this may be, that this cell  $1d^1$  divides more unequally than the others, and the principal cause of the delayed cleavage of the posterior basal must, therefore, be sought in whatever agency gives rise to the unequal division of  $1d^1$ . In *Planorbis* the division of this cell is no more unequal

than that of the others in the same tier, and the basal cell of the cross which arises from it, being, therefore, of the same size as the other basals, divides approximately at the same time. We have, therefore, in the different character of the division of  $1d^1$  in *Crepidula* and *Planorbis*, the beginning of the difference in the course of development of the posterior arm of the cross in the two forms. We may be unable to explain the differential character of this cleavage of  $1d^1$ . Why one out of four protoplasmic cells, identical, so far as we can determine, in size and structure, should divide much more unequally than the others is certainly not apparent. Nevertheless it is desirable to find the precise point where the histories of structures in different forms begin to diverge, although we are unable to discover the antecedent phenomena which give the direction to the different lines of divergence. The earlier cleavage of the posterior tip cell in *Crepidula* is probably connected with its larger size. Its cleavage is radial, and each of the daughter-cells divides again in a radial direction, giving rise in all to six cells in the posterior arm. The cause of these successive longitudinal divisions may possibly be the growth of the posterior trochoblasts and the cells lying below them, which at this period have reached a considerable size. The enlargement of these cells would naturally subject the arm to pressure at the sides and give the cells a longitudinal elongation. A comparison of Conklin's Fig. 49 with Fig. 53 shows that the trochoblasts in the latter figure are larger, and the basal and tip cells longer and narrower. The shape of the tip cells just before their division is not figured, but it is probable that they have become more or less compressed like the basals, after the stage shown in Fig. 51. However, after their division they have been narrowed to about one-half their former diameter (Fig. 53). The posterior tip cell in *Planorbis* has quite a different history. In the first place it is no larger than the tip cells of the other arms, but the marked difference it presents from the corresponding cells in *Crepidula* is that it never divides. It increases enormously in size and becomes transparent. Its shape changes entirely; at first it is elongated transversely to the arm of the cross; gradually, as it enlarges, it becomes

elongated in the opposite direction and takes part in the formation of the head vesicle. The elongation of this cell goes hand in hand with the growth of the posterior trochoblasts, whose increase in size would naturally subject it to a lateral pressure. The peculiar history of this cell is evidently correlated with the large size which the head vesicle attains in this form. It affords another case of precocious specialization of function such as occurs in the trochoblasts. In fact, the fate of this cell and the posterior trochoblasts is identical, as they all go to form the same organ. The head vesicle in *Planorbis* develops early, and reaches a much larger size than it attains in *Crepidula*. The posterior tip cell becomes differentiated at an earlier period and stops dividing. In *Crepidula* it divides twice, perhaps many times, and the products of these divisions remain for a long time apparently little modified. Their precise fate is uncertain; probably some of them, at least, enlarge and enter into the formation of the head vesicle, as in *Planorbis*.

The other tip cells divide twice in *Crepidula*, forming a row of four small cells lying across the tips of the arms of the cross. All of these cells, accordingly, go into the upper row of cells, forming the prototroch. Except in the anterior arm of the cross, the tip cells in *Planorbis* do not divide at all. The lateral tip cells, like the posterior one, enlarge, become transparent, and go to form a part of the head vesicle. There is probably no other group of cells in these two forms which present such marked differences of behavior as the tip cells of the cross. In *Crepidula* they are at first very small and of unequal size; they grow very little and divide several times. In *Planorbis* they are at first quite large and of equal size; they grow quite rapidly, and, with the exception of the anterior one, never divide at all. In *Crepidula* those of the anterior and lateral arms go into the prototroch; in *Planorbis* only the anterior one goes into the formation of this organ, and this cell, as far as could be determined, undergoes only one division.

The next cleavage, after the stage to which the history of the cross has been traced, occurs in the four cells lying in the angles between the arms. These divisions are bilateral; in the anterior pair of cells the left cell divides dextrotropically, the right



cell laeotropically; in the posterior pair the cleavage of the right cell is dextrotropic, that of the left one laeotropic. The two cells in the anterior arm,  $1b^{1,2,1,2,1}$ ,  $1b^{1,2,1,2,2}$ , next divide, the right cell in a dextrotropic, the left in a laeotropic direction. The posterior cells on either side resulting from this division come to lie to the outside of the small, outer, intermediate cells, so that they no longer lie in contact with the trochoblasts (Pl. XX, Fig. 42). (Compare the cleavage of  $1b^{2,2,1}$  and  $1b^{2,2,2}$  in *Crepidula*.) Next the anterior median cell,  $1b^{1,2,2}$ , divides transversely, and each of the daughter-cells divides bilaterally, in the same direction as that of the pair of cells just described.

At this period the general form of the cross has undergone marked changes. The cells of the posterior arm have increased greatly in size and become distorted in shape. The posterior trochoblasts enlarge unequally, and the posterior arm may be pushed either to the right or the left. The posterior tip cell first increases in size; afterward, all the cells lying in front of it also enlarge. The enlargement of cells does not stop with the cells of the posterior arm, but the four apical cells increase in size also (Pl. XX, Fig. 42). The central and anterior portions of the cross are pushed forward more rapidly than the lateral arms, which thus appear to be bent backwards. The darkly staining portion of the cross has now the shape of a *V*, with the apex pointing anteriorly. The two anterior tip cells, as the cross is pushed forward, come to lie more and more nearly side by side, and, finally, they become arranged transversely across the tip of the cross. As they were originally somewhat oblique, this arrangement would very naturally result from a pressure due to the forward rotation of the apical cap of cells.

The process of enlargement of cells extends forward to the cells lying in front of the apicals, and eventually forms a tract of large clear cells, which separates the two halves of the cross and reaches the prototroch in front. The basal cell of the anterior arm of the cross, since it lies in the median line, takes part in this enlargement of cells; as it increases in size, it becomes pushed forward, and the cells in front of it, which lie symmetrically on either side of the median axis of the arm, are forced aside. The fate of this cell has been carefully traced, as



it has a peculiar and most interesting history. At first it lay at the base of the anterior arm of the cross. When the cells  $1b^{1,2,1,2,1}$  and  $1b^{1,2,1,2,2}$  divide, this cell comes to lie between the cells produced by these divisions, and soon it is seen further forward, between the posterior pair of cells arising from the third cell of the arm,  $1b^{1,2,2}$ . Pl. XX, Fig. 42, shows this cell where the anterior cells are in contact, and Pl. XX, Fig. 48, shows it pushed still further forward, until it has forced the cells  $1b^{1,2,2,1,1}$  and  $1b^{1,2,2,2,1}$  to either side and come into contact with the tip cells. Its journey does not end here, but it apparently pushes aside the tip cells as well, and comes in contact with the cells of the second quartette, which lie below them (Pl. XX, Fig. 46). *This cell has, therefore, pushed its way through the split anterior arm from the base to the tip.* In later stages it becomes much elongated transversely, and forms a part of the prototroch. The origin of this upper median cell of the prototroch was for a long time a puzzling problem. I have fortunately found all stages of the process by which it travels through the middle of the anterior arm of the cross to its definitive position.

The further history of the cells of the cross is very difficult to follow. The cells in the center enlarge unequally in different cases, and the position of the cells is altered considerably by this process, which makes it very difficult to follow their lineage. I have observed a bilateral division of the cells  $1c^{1,2,1,1,1}$  and  $1a^{1,2,1,1,1}$ , the lines of division converging anteriorly. Both products of this division then divide at right angles to the preceding cleavage.

#### *The Second Quartette.*

The cleavage of the cells of the second quartette has already been traced to a stage in which there are four cells in each quadrant. The two middle cells in each quadrant, which are larger than the upper and lower cells, are the first to divide; the division of both cells in each pair is laetotropic, the cleavage of the right cell occurring a little before the left. These divisions occur between the fifty-two and the sixty-four cell

stages; the lower cells resulting from these divisions are somewhat larger than the upper ones. When the egg contains about seventy-five cells, the lower cell in each quadrant divides, generally in a laeotropic direction, but the direction of the cleavage does not appear to be constant. Pl. XIX, Fig. 32, shows that the division of  $2a^{2.2}$  was probably dexiotropic, but shifting of the position of cells has of course to be allowed for. The cells  $2a^{2.2}$ ,  $2b^{2.2}$ , and  $2c^{2.2}$ , previous to their division, have become more and more flattened in a direction transverse to the vertical axis of the egg. After their division their daughter-cells become flattened still more in the same direction; one of these usually lies above the other, which alone comes in contact with the entomeres; sometimes they lie obliquely, especially in the  $a$  and  $c$  quadrants, the outer cell touching the entomeres by a small portion of its boundary. These cells form the anterior and lateral boundaries of the blastopore, the cells of the third quartette lying at the angles. It will be convenient to apply the term "stomatoblasts" to these cells,  $2a^{2.2}$ ,  $2b^{2.2}$ , and  $2c^{2.2}$ , and their derivatives, as they form a part of the margin of the blastopore, and later take part in the formation of the stomodaeum. The term "stomatoblasts," however, was used by Wilson to designate the cells  $2a^2$ ,  $2b^2$ , and  $2c^2$ , and their derivatives, which in *Nereis* form a ring of cells around the blastopore. In *Planorbis* the cells  $2a^{2.1}$ ,  $2b^{2.1}$ , and  $2c^{2.1}$  never come in contact with the blastopore, and probably do not take part in the formation of the stomodaeum, so that the term "stomatoblasts" would hardly seem applicable to them. The term "stomatoblasts" is used, therefore, to designate only the lower product of the division of the cells which correspond to the stomatoblasts of Wilson. In *Nereis* the division of the cells  $2a^2$ ,  $2b^2$ , and  $2c^2$  is transverse to the polar axis and all of the products of the cleavage border on the blastopore. In *Planorbis* these cells divide in nearly the opposite direction, so that  $2a^{2.1}$ ,  $2b^{2.1}$ , and  $2c^{2.1}$  lie above the ventral cells and never come into relation with the entomeres. The same cells divide into an upper and a lower moiety in *Unio*, *Neritina*, *Umbrella*, *Crepidula*, *Limax*, and *Ischnochiton*. In none of these forms, except *Ischnochiton*, has the cleavage of  $2a^{2.2}$ ,  $2b^{2.2}$ ,  $2c^{2.2}$  been described. The cell

$2d^{2,2}$  differs in shape and in the direction of its cleavage from the other cells of the same tier. Before it divides, it becomes elongated in a vertical instead of a horizontal direction. It divides laetotropically, its daughter-cells lying obliquely side by side, with their long axes nearly radial. Later, owing to the approach of the large cells  $3a^2$  and  $3d^2$ , the cell  $2d^{2,2}$  becomes pushed upwards, losing its connection with the entoderm when these cells meet in the middle line.

The next divisions in this quadrant occur in the cells  $2a^{1,2,2}$ ,  $2a^{2,1,2}$ , etc. The direction of the cleavage is nearly radial, but slightly dexiotropic. The corresponding cleavages in the  $b$  quadrant are delayed until a later period. The upper pair of cells in the quadrants  $a$ ,  $c$ , and  $d$  next divide, the right cell laetotropically, the left dexiotropically. The cells from the adjacent arms of the cross push under the trochoblasts from either side and often meet each other, thus separating the trochoblasts entirely from the cells of the third quartette (Pl. XIX, Fig. 32). Owing to the forward rotation of the apical cap of cells, the cells  $2b^{1,2,1}$  and  $2b^{2,1,1}$  become pushed apart, so that they come to lie on either side, instead of above the cells  $2b^{1,2,2}$  and  $2b^{2,1,2}$  (see Pl. XX, Fig. 39). The cleavage of the tip cell  $2b^{1,1}$  has already been mentioned; its two daughter-cells, owing to the rotation of the apical pole, come to lie side by side and in contact with the cells  $2b^{1,2,2}$  and  $2b^{2,1,2}$ . The cells  $2b^{1,1,1}$ ,  $2b^{1,1,2}$ ,  $2b^{1,2,1}$ ,  $2b^{2,1,1}$ ,  $2b^{1,2,2}$ , and  $2b^{2,1,2}$  all become large and clear and enter into the formation of the prototroch. In the anterior quadrant the three upper cells of the four, therefore, enter into the prototroch as in the annelids, and their products undergo, I believe, no further divisions beyond the stage just described. The cell  $2b^{2,2,1}$  divides horizontally; this is the last cleavage in this quadrant that could be observed. The cleavage of the second quartette has been followed to a stage in which there are eleven cells in each of the quadrants  $a$ ,  $c$ , and  $d$ , and ten in the anterior, or  $b$ , quadrant. After this stage the divisions of this quartette become very difficult to follow. I have seen a nearly horizontal cleavage of  $2c^{1,2,2,1}$ , and have been able to recognize the cells of this quartette when there are fifteen cells in each quadrant, but only hypothetical derivations of these

cells can be given. The group of cells in the *b* quadrant of the second quartette is shorter and broader than the other groups. This is doubtless due to the forward rotation of the apical pole of the egg, which would exert a vertical pressure on this group of cells. The slower cleavage in this group may be also due, in a certain degree, to the same cause, though it is probably correlated with the fate of these cells. The portion of this quadrant which does not go into the prototroch forms only an exceedingly small portion of the body of the embryo, being used, as far as could be determined, to form a part of the stomodaeum.

The early cleavages of the second quartette in *Planorbis* are very similar to those of other gasteropods. Kofoed has traced the cleavage of this quartette in *Limax* to a stage in which there are four cells in each quadrant, and these cells agree almost exactly in relative size and arrangement with those in *Planorbis* at the same stage. Blochmann ('82) figures a stage in which there are seven cells of this quartette in each quadrant, and their arrangement is very similar to that found in *Planorbis*. Heymons has traced the lineage of this quartette to a stage in which there are eleven cells in each quadrant, but he does not describe the direction of the cleavage of the tip cells. Conklin has followed the cell lineage of the second quartette to a stage in which each quadrant contains eleven cells, and has traced the cleavage of the tip cells somewhat beyond this point. The agreement of the direction of the divisions of this quartette in *Planorbis* with those in the above forms is quite close, but there are some minor differences. In general, we may say that the cleavages are more nearly radial than in the two last forms. For instance, the cleavages of  $2a^{2.1.2}$  in *Crepidula* and  $2a^{1.2.2}$  in *Umbrella* are nearly horizontal, while in *Planorbis* they are nearly radial. All the cells lying between the tip cell and the lowest cell of the group have, at a stage when there are eleven cells in each quadrant, exactly the same lineage in *Umbrella*, *Crepidula*, and *Planorbis*. In the first two forms the cleavage of the tip cell has occurred, while the lowest cell,  $2a^{2.2}$ ,  $2b^{2.2}$ , etc., remains undivided; in *Planorbis* the lowest cell has divided, while the tip cell, except in the anterior quadrant,



remains entire. In both Umbrella and Crepidula the divisions of the second quartette, as far as they have been traced, are, with the exception of the cleavage of the tip cell in the latter form (and, possibly, also in the former), perfectly similar in every quadrant. This may be due to the fact that the forward rotation of the apical pole occurs later in these forms. The fact that when this rotation is delayed the similar character of the division of the quadrants of the second quartette is maintained for a longer period, lends additional support to the view that in Planorbis the delayed cleavage in the anterior quadrant is causally connected with this rotation. The early beginning of this rotation in Planorbis, caused as it is by the more rapid growth of the posterior trochoblasts and the posterior arm of the cross, may be viewed as a result of the early formation of the head vesicle. This structure develops early in Planorbis, and reaches a larger size than in Crepidula or Umbrella. The different behavior of the cells of the anterior quadrant of the second quartette in these different gasteropods may thus be considered a sort of indirect effect of the different degrees of development which the head vesicle attains in these forms.

#### *The Mesoblastic Bands.*

The mesoblastic bands in Planorbis have been fully described by Rabl, who was the first to derive the mesoderm in the gasteropods from a single cell. A division occurs, however, after the first cleavage of the primary mesomere, which it seems that Rabl overlooked. After the two mesomeres have come to lie entirely in the cleavage cavity, each buds off at the anterior end a minute clear cell, which is often quite difficult to observe. The spindles are inclined slightly towards each other at their anterior ends, and the small cells that arise lie almost in contact with each other. The next cleavage of the mesomeres is horizontal and equal, and at right angles to the preceding division. There thus result an inner and an outer pair of large mesodermic cells, which are figured by Rabl in Figs. 17*a* and 17*b*. The inner pair of cells are the mesoblastic teloblasts. The next cleavage of the teloblasts is in the same direction as

before, but this time the division is unequal and gives rise to a small cell lying between the middle and outer cells. The next cleavage occurs in the outer pair of cells and in the same direction as the preceding division. In fact, all the divisions in the mesoblastic bands are henceforth in the same direction, until a considerably later period of development. At the time when there are three cells in each band the mesomeres form a concave row of cells in the posterior half of the egg. As the bands lengthen by teloblastic budding, they assume the shape of a horseshoe. Later they become resolved into scattered cells.

*The Third Quartette and the Secondary Mesoblast.*

At the stage in which the egg contains forty-nine cells the cells of the third quartette are eight in number, arranged in four vertical pairs, lying over the angles between the cells of the fourth quartette. The first cleavage of this quartette forms a transition from the spiral to the bilateral type, and the subsequent cleavages show a bilateral character in a more marked degree. At nearly the same time the *lower* pair of cells,  $3b^2$ ,  $3c^2$ , in the two anterior quartettes, and the *upper* pair of cells,  $3a^1$ ,  $3d^1$ , in the posterior quadrants, divide in a nearly horizontal direction into equal moieties. Later, at about the sixty-four-cell stage, the upper pair of cells in the anterior quadrants,  $3b^1$ ,  $3c^1$ , divide in the same direction as the lower pair. The lower pair of cells in the two posterior quadrants,  $3a^2$ ,  $3d^2$ , remain undivided until a much later stage. In each of the two anterior quadrants there are now two pairs of cells, the one pair lying directly above the other. In each of the posterior quadrants there is a pair of cells lying over a large undivided cell. It is easy to orient the egg from the lower pole at this stage by the bilateral arrangement of these cells. Definite spiral cleavage, which appears in such a marked way in the early divisions of the first and second quartettes, seems entirely absent in the third. The first cleavage is radial, and all the succeeding divisions appear to be bilaterally symmetrical with reference to the median plane of the future animal.



The next divisions in this quartette occur in the cells  $3a^{1,1}$ ,  $3a^{1,2}$ ,  $3d^{1,1}$ ,  $3d^{1,2}$ , on the posterior side of the egg, and in  $3b^{2,1}$ ,  $3b^{2,2}$ ,  $3c^{2,1}$ ,  $3c^{2,2}$ , on the anterior side. These divisions are radial and similar in character, each cell budding off a small cell toward the vegetal pole. There thus arise four pairs of small cells, the two anterior pairs lying in the angles between the entomeres on either side of the median plane, the two posterior pairs lying above the large cells,  $3a^2$  and  $3d^2$ . The upper pair of cells in each of the four quartettes next divide in the same direction as before, forming a vertical series of four pairs of cells in the anterior quadrants, and a similar series of three pairs of cells above the large cells,  $3a^2$  and  $3d^2$ , in the posterior quadrants. The third quartette now contains thirty cells, eight in each anterior quadrant, and seven in each posterior one.

The two pairs of cells,  $3b^{2,1,1}$ ,  $3b^{2,2,1}$ ,  $3c^{2,1,1}$ , and  $3c^{2,2,1}$ , become pushed in towards the cleavage cavity and become partly covered by the surrounding cells. They divide in a nearly horizontal direction, and their daughter-cells become pushed into the cleavage cavity still further. They form an irregular row of four small cells lying above the pairs of small cells in the angles between the entomeres. The period of their division is quite variable. In one case (Pl. XIX, Fig. 27) a division has evidently occurred in  $3c^{2,2,1}$  before the upper cells have divided, but this does not usually occur. In Pl. XIX, Fig. 33,  $3c^{2,2,1}$  has divided, while the cell lying beside it is entire, as are also the corresponding cells in the *b* quadrant. The lower products of the division of the uppermost cells in the anterior quadrants,  $3b^{1,1,2}$ ,  $3b^{1,2,2}$ ,  $3c^{1,1,2}$ ,  $3c^{1,2,2}$ , divide in a nearly radial direction. Pl. XIX, Fig. 33, shows  $3c^{1,1,2}$  dividing, while the cleavage in  $3c^{1,2,2}$  is completed. The number of cells in each anterior quadrant is now twelve; the small cells in the angles between the entomeres have remained undivided since their origin; above this pair are the four cells, which are partly sunk into the cleavage cavity, arranged in a transverse row, and above these again are three pairs of cells in a vertical series. The number of cells in each posterior quadrant is still seven, and the whole number of cells in the third quartette is thirty-eight. The entire egg contains at this period about 150 cells. The four cells in each

of the anterior quadrants, which lie partly pushed into the cleavage cavity, finally lose connection with the ectodermic wall and come to lie in the blastocoel. The cells from the two sides nearly meet, forming a curved row of cells, the posterior ends of which nearly meet the anterior ends of the mesoblastic bands, which curve forward from the posterior side of the egg. The anterior row of cells forms what Wierzejski calls the secondary mesoderm. As far as can be judged from the very brief description of this process in Wierzejski's preliminary paper, the formation of the secondary mesoderm in *Physa* is very similar to, if not identical with, its formation in *Planorbis*. The first cleavage of the cells of the third quartette in *Physa* is radial, as in *Planorbis*, and the lower cell in the two anterior quadrants divides horizontally into a right and left cell. The cleavage of the upper cell is not described. The next division of the lower cells is the same as in *Planorbis*, each giving off a small cell toward the vegetal pole. The second pair of cells,  $3b^{2.1.1}$ ,  $3b^{2.2.1}$ ,  $3c^{2.1.1}$ ,  $3c^{2.2.1}$ , divide again in a radial direction, the upper cells going to form mesoblast; the fate of the lower cells was not determined. In *Planorbis* this cleavage is nearly transverse, the outer cells being somewhat higher than the inner ones, and probably corresponding to the upper cells (*Mutterzellen*) in *Physa*. If the lower cells,  $3b^{2.1.1.2}$  and  $3b^{2.2.1.2}$ ,  $3c^{2.1.1.2}$ ,  $3c^{2.2.1.2}$ , whose fate Wierzejski did not determine, also form secondary mesoblast, the cell origin of the secondary mesoblast in the two forms would be identical.

The last cleavages observed in the cells of the third quartette were those of the large cells,  $3a^2$  and  $3d^2$ . These divide bilaterally and in a nearly horizontal direction, a small cell being given off from each at the outer end. Later these cells give off another small cell in the same direction as before. After these two divisions these cells are considerably reduced in size, but at the time gastrulation begins they form a rather conspicuous pair of cells, lying behind the nearly circular group of entomeres. The third quartette at this stage is composed of forty-two cells.

*General Considerations on the Secondary Mesoblast.*

The origin of the secondary mesoblast in the Mollusca is a subject to which, for many reasons, considerable interest is attached. We possess, however, at present very few accounts of the process by which the secondary mesoblast arises. The first case in which a double origin of the mesoderm has been carefully and accurately traced is that of *Unio*, studied by Dr. Lillie. In this form both the primary and secondary mesoblast are segregated at an early period. The primary mesoblast arises from  $4d$ , as in other mollusks; the secondary or "larval mesoblast," as it is called by Lillie, arises asymmetrically from a cell of the second quartette,  $2a^2$ , on the left side of the egg. This cell is gradually overgrown by the surrounding cells, and, after budding off two or three small cells to the surface, comes to lie entirely in the blastocoel. Although the secondary mesoblast arises asymmetrically, it afterwards becomes disposed in a symmetrical manner by the migration, apparently, of some of the cells to the opposite side of the egg. The larval mesoblast forms a kind of mesenchyme, which gives rise to certain larval structures, which disappear in later development, and its early segregation appears to be correlated with the early development and importance of these organs in larval life. It is a significant fact that the cell from which the larval mesoblast arises is larger than the corresponding cell on the other side of the egg.

Secondary mesoblast was discovered later in *Crepidula* by Conklin. It was found to arise near the edge of the blastopore in the three quadrants,  $a$ ,  $b$ , and  $c$ , in which no other mesoblast was produced. The exact cell origin of this mesoblast Conklin was unable to trace, owing to the large number of cells in the egg at that stage, but, from the position of the mesoblast cells, it was shown to have arisen from the cells of the second quartette. There is an anterior mesoblast cell in the  $b$  quadrant, and a right and left cell bilaterally placed in the  $a$  and  $c$  quadrants. The  $d$  quadrant produces no secondary mesoblast unless at a very much later period. The mesoblast in *Crepidula* arises, therefore, in each of the four quadrants.

The secondary mesoblast in *Physa*, according to Wierzejski,

and in *Planorbis*, according to my own observations, has yet a different origin, arising from the cells of the third quartette in the two anterior quadrants. It arises, as in *Crepidula*, at a late period of cleavage, and its origin is likewise bilateral. As the cells of the third quartette are arranged symmetrically on either side of the median axis of the egg, its origin from three quadrants could not be bilaterally symmetrical. It is quite certain that, in *Planorbis* at least, no secondary mesoblast arises from the posterior quadrants, unless at a very much later period of development.

Several cases have been pointed out by Lillie, among accounts of the embryology of the lamellibranchs, where the figures of the authors show strong evidence of the existence of secondary mesoblast. The figures of *Cyclas* by Ziegler and Stauffacher, of *Teredo* by Hatschek, of *Anodonta* by Goette and by Schierholz, of *Ostrea* by Horst, show mesoblast cells in the early stages of gastrulation that could scarcely have arisen from the pole cells. In all these genera, cells are figured in front of, as well as behind, the blastopore. There are also similar cases in papers on other groups of mollusks. Kowalevsky's figure of a sagittal section of the larva of *Dentalium* shows a large mesoblast cell in the blastocoel at either end of the gastrula. And similar indications of secondary mesoblast are shown in Fol's figure of a sagittal section of the larva of *Firoloides*.

The case of *Paludina vivipera* is an interesting one in this connection. It is one of the few points of agreement, among those who have worked on the form, that mesoblastic pole cells do not occur. Tönniges finds that, in this form, mesoblast is produced from certain cells lying in front of the blastopore. If we accept Tönniges's account, the formation of the mesoblast in *Paludina* would seem to correspond to the formation of the secondary mesoblast in other forms.

The researches of Eisig and Wilson on the development of annelids suggest that the occurrence of secondary mesoblast may be typical for both annelids and mollusks, and indicate another striking point of agreement to the many which exist between the methods of cleavage of these groups. At the same time they serve to connect more closely the cleavage of anne-



lids and mollusks with that of the polyclades, in which the mesoderm has a radial origin from one or more quartettes of micromeres. Until more is known, however, of the origin, and especially of the fate, of the cells which have been called secondary mesoblasts, it must remain uncertain whether there is any true homology between these cells and the mesoblast of the polyclades, although such a comparison naturally suggests itself. The subject is one of considerable interest from the standpoint of phylogeny, and the reader may be referred for suggestive discussions of the problem to the papers of Conklin ('97), Wilson ('98), and Eisig ('98).

#### *The Entomeres.*

The egg of *Planorbis* is peculiar among the eggs of mollusks, in that the entomeres are of small size and undergo numerous divisions before the beginning of invagination. The fourth quartette consists of cells which greatly exceed in size the four small cells at the vegetal pole. The three cells of this quartette which form entoderm, *4a*, *4b*, and *4c*, divide horizontally when the egg contains about fifty-six cells. The six cells resulting from this cleavage are arranged in the form of a horseshoe about the four cells in the center, the opening between the ends of the curve being on the posterior side of the egg. The next cleavage occurs when the egg contains about ninety cells. Each of the six cells of the fourth quartette divides in a nearly radial direction. These divisions are, however, slightly oblique, and are bilaterally symmetrical with respect to the median plane of the egg. The divisions of the cells on the right side of the egg are slightly laeotropic, while those on the left side are slightly dexiotropic. These divisions are followed by a cleavage of the three small cells, *A*, *B*, and *C*, at the vegetal pole, forming a fifth quartette. The cell *D*, which is the smallest of the group, does not divide; nor have I been able to observe its cleavage at any subsequent stage, although it could be recognized after the process of gastrulation had made some progress. It is quite an exceptional fact that the cell *D*, which, in many forms, is the largest cell of the egg, should, in *Planorbis*, be the least in size

of all the cells. From the twenty-eight-cell stage until the period of gastrulation it has remained without a single division. The cleavage of the central cells is bilateral; the cell *B* divides radially; the cleavage of *A* is laeotropic; that of *C* dextrotropic. These divisions are followed by an equatorial cleavage of the cells of the fourth quartette. The derivatives of *4b* divide first, but the cleavage of the cells in the other two quadrants soon follows. There are, after these divisions are completed, twenty-four cells of the fourth quartette and three of the fifth; these, with the four small cells at the vegetal pole, make a total of thirty-one entomeres. The entoderm cells at this stage are of small and about equal size, and are easily distinguished from the surrounding ectoderm cells by their yellow color. Their number is increased somewhat before gastrulation has progressed very far, but I have not attempted to follow their cleavage beyond the point just described.

Small spheres of an albuminous substance gradually accumulate in the entoderm cells and become quite numerous near the period of gastrulation. These spheres stain very darkly in haematoxylin and make observation of the nuclei very difficult. At their first appearance, these dark bodies are found also in the ectoderm; but, as development proceeds, they become more and more confined to the entodermic cells. The staining reaction of these bodies is much like that of the surrounding albuminous matter in which the embryo floats, and it is very probable that they are simply masses of albuminous substance that has been ingested by the cells and has not been chemically transformed. After invagination certain of the entodermic cells increase enormously in size, becoming filled with a transparent, yellowish substance that is little affected by stains. Rabl has shown that in the end of the cells turned toward the enteron there are masses of deeply staining substances which he regards as material which has been absorbed by the cells, but not completely transformed into the yellowish substance which fills the greater part of the cell. It is doubtless the same material that forms the darkly staining bodies in the egg.

The ingestion of albumin occurs in a similar manner in the egg of *Limax*, and has been described by Meisenheimer, whose











figures show very clearly the history of the process. The deeply staining bodies occur in all the cells at an early period, even in the sixteen-cell stage, and increase in number as the development proceeds. The cells of the ectoderm take in the albumin more rapidly than in *Planorbis*; but, finally, this function is transferred entirely to the cells of the entoderm. The process of digestion of albumin, by which the egg is nourished and enabled to grow, is carried on at first with equal facility by all the cells of the egg. This process soon predominates in the entomeres, although, for a time, it is carried on more rapidly by all the cells. Finally, the ingestion of albumin is relegated to the cells of the enteron, which have become specialized for digestive purposes, and the other cells of the egg no longer share this function.

*The Rudiments of the Cerebral Ganglia and Eyes.*

The two portions of the cross, which are separated by the median apical plate, form the regions which give rise to the cerebral ganglia and eyes. These two rudiments, in eggs stained with silver nitrate, appear as dark masses surrounded on every side by large and very transparent cells. The tip cells of the lateral arms, and the cell lying immediately above them, do not enter into the formation of these masses, but increase in size and go into the head vesicle. With the exception of these two cells in each arm, all the cells in the lateral arms of the cross, the cells of the anterior arm, except the tip and basal cell, and the central region of the cross, except the four apicals and the two cells lying in front of them, enter into the formation of these two rudiments. The composition of these two patches of cells when they are definitely marked off may be seen in the following table :

LEFT RUDIMENT.		RIGHT RUDIMENT.	
$Ia^{1.1.2.1}$	$Ia^{1.2.1.1.1.3}$	$Ic^{1.1.2.2}$	$Ib^{1.2.2.1.1}$
$Ia^{1.1.2.2}$	$Ib^{1.1.2.2}$	$Ic^{1.2.1.1.2}$	$Ib^{1.2.2.1.2}$
$Ia^{1.2.1.1.2}$	$Ib^{1.2.1.2.2.1}$	$Ic^{1.2.1.2.1}$	$Ib^{1.2.1.2.1.1}$
$Ia^{1.2.1.2.1}$	$Ib^{1.2.1.2.2.2}$	$Ic^{1.2.1.2.2}$	$Ib^{1.2.1.2.1.2}$
$Ia^{1.2.1.2.2}$	$Ib^{1.2.2.2.1}$	$Ic^{1.2.1.1.1.1}$	$Ia^{1.1.2.1}$
$Ia^{1.2.1.1.1.1}$	$Ib^{1.2.2.2.2}$	$Ic^{1.2.1.1.1.2}$	$Ia^{1.1.2.2}$

There are the same number of cells in each of the two rudiments which, as far as the size and position of their component cells is concerned, show a perfect bilateral symmetry. Yet the derivation of the cells on the two sides does not exactly correspond. The right rudiment contains cells from three quadrants, while the left contains cells from but two. This is because the intermediate cells from the *d* quadrant lie to the right of the median axis of the egg. The posterior arm of the cross, which is composed of cells of this quadrant, takes no part in the formation of these structures. The two products of the cleavage of the intermediate cell and the apical  $1d^{1,1,1}$ , which enters the apical plate, are the only cells of the first quartette in the *d* quadrant which do not go to form the head vesicle.

The cells forming the cerebral rudiments multiply with great rapidity. The areas become thickened and a proliferation of cells occurs which gives rise to the cerebral ganglia. The early appearance of these rudiments was observed by Rabl, who designated them a bilobed apical plate (Scheitelplatte). The further history of the fundaments of the cerebral ganglia may be followed in Rabl's paper. The areas above described are not exclusively employed in the formation of the cerebral ganglia and eyes. The cells become so numerous before differentiation begins that it is impossible to trace the cell origin of the different structures arising from them. As the eyes arise at the outer sides of the cerebral ganglia, it is quite certain that they are formed from cells derived from the lateral arms of the cross. Whether this can be said also of the tentacles is uncertain.

#### *The Apical Plate.*

The term "apical plate" has been applied by Conklin to a median belt of large, clear cells in *Crepidula* extending from the apical sense organ to the prototroch. It is composed of seven cells which become covered by fine cilia and remain for a long time undivided, while the neighboring cells rapidly multiply. In *Planorbis* there is a median belt of large cells extending from the head vesicle to the prototroch, and which, from its similarity to the apical plate in *Crepidula*, I have designated by



the same name. The peculiar apical sense organ in *Crepidula*, I am satisfied, does not occur in *Planorbis*. In the former genus the four cells which form this organ remain of small size, acquire a tuft of long cilia, and become united later with a pair of nerve cords which arise from the cerebral ganglia on either side. The existence of such a structure in a molluscan larva is a striking mark of relationship with the annelid trochophore. The presence of this organ in the larva of *Planorbis* might naturally be looked for, but the four apical cells which form this organ in *Crepidula* become in *Planorbis* very much enlarged and thinned out and form a part of the apical plate. The cells which compose this plate are six in number, the four apical cells just mentioned and a pair of cells lying in front of these. Of the origin of this pair of cells I am not entirely certain, but I think they are the cells  $1b^{1.1.2.1}$  and  $1c^{1.1.2.1}$ . These cells arose from the divisions of the intermediate cells lying in the angles between the arms of the cross. The cell lineage of the apical plate in *Planorbis* may be expressed as follows:

$$\left\{ \begin{array}{l} \text{apical cells } 1a^{1.1.1}, 1b^{1.1.1}, 1c^{1.1.1}, 1d^{1.1.1} \\ \text{intermediate cells } 1b^{1.1.2.1}, 1c^{1.1.2.1} \end{array} \right.$$

The anterior end of the apical plate is limited by the upper median cell of the prototroch, and its posterior boundary is formed by the basal cell of the posterior arm of the cross.

#### *The Cell Lineage of the Head Vesicle.*

Owing to the fact that the cells composing the head vesicle in *Planorbis* are few in number and of large size, I have been able to determine the exact lineage of all the components of this structure. The head vesicle may be said to first appear when the posterior trochoblasts and the cells of the posterior arm of the cross begin to enlarge. This enlargement, which begins before the 100-cell stage, causes the upper pole to move toward the anterior side of the egg. The tip cells of the lateral arms of the cross enlarge rapidly, and, at a later period, the cells  $1a^{1.2.2}$ ,  $1c^{1.2.2}$ , lying just above the tip cells, also enlarge. All of these cells, owing to the anterior rotation of the upper pole

of the egg, come to lie behind the masses of cells which form the rudiment of the cerebral ganglia.

The cells composing the head vesicle are given in the following table :

Cells of the posterior arm of the cross	$\left\{ \begin{array}{l} 1d^{1,2,1} \\ 1d^{1,2,1,2} \\ 1d^{1,2,2} \\ 2d^{1,1} \end{array} \right.$
Posterior trochoblasts	$\left\{ \begin{array}{l} 1d^{2,1} \\ 1d^{2,2} \\ 1a^{2,1} \\ 1a^{2,2} \end{array} \right.$
Cells of the lateral arms of the cross	$\left\{ \begin{array}{l} 1a^{1,2,2} \\ 2a^{1,1} \\ 1c^{1,2,2} \\ 2c^{1,1} \end{array} \right.$

The number of cells in the head vesicle is twelve, of which nine belong to the first and three to the second quartette. The area of the head vesicle, when it reaches its maximum size, is fully equal to that of the rest of the embryonic body. The cells composing it are very thin and transparent and of relatively enormous size. All of the cells which make up the head vesicle were present in the egg after the division of the cells  $1a^{1,2}$ ,  $1b^{1,2}$ , etc., which occurs at about the 64-cell stage, and only one cell which is destined to form a part of the structure, *viz.*,  $1d^{1,2,1}$ , undergoes division after this period. The cells, indeed, become so exceedingly thin in later stages that it is difficult to see how their division could be effected. What becomes of these cells when the head vesicle disappears is uncertain.

#### *The Cell Lineage of the Prototroch.*

It has been long known that the velum in the pulmonate gastropods is a rudimentary structure. It was first noticed by Vogt, and has been described since in various pulmonates by Rabl, Fol, and Lankaster. The velar lobes, which are such a characteristic feature in the so-called veliger stage in other mollusks, are absent in the pulmonates, and all that can be said to correspond to the velum is a double row of ciliated cells extend-

ing from the ventral side of the body, immediately in front of the mouth, towards the dorsal side of the embryo. This row of cells has been called the prototroch, from its similarity to that structure in the annelid trochophore. Owing to the marked resemblance of this structure in the larvae of two such distinct groups as mollusks and annelids, considerable interest is naturally attached to a comparison of its cell origin in these forms.

The cell origin of the prototroch was first determined in the annelids by E. B. Wilson, who discovered that in *Nereis* the four cells,  $1a^2$ ,  $1b^2$ , etc., which he called the trochoblasts, gave rise to this organ. Later, the origin of the prototroch in several annelids has been carefully studied by Dr. A. D. Mead, with the result that the cell origin of this organ was shown to be identical in every case. In *Amphitrite* and *Clymenella* the cell lineage of the prototroch was carried out in detail to a late stage, but the other forms studied agree with these as regards the protoblasts of this organ as far as their cleavage was observed. In both *Amphitrite* and *Clymenella* the prototroch arises from the four trochoblasts, which divide twice, forming sixteen cells, and three of the cells of the second quartette in each of the three quadrants, *a*, *b*, and *c*. Thus three out of the four cells of the second quartette in each of these three quadrants go to form the prototroch. Mead argues that in *Nereis*, also, the prototroch arises in the same way, although Wilson's derivation of this organ differs from Mead's as regards the fate of the upper cells of the second quartette. Child's account of the formation of the prototroch in *Arenicola* agrees, point for point, with Mead's, and Mr. Treadwell's observations in *Podarke* indicate a similar origin of the prototroch in that form (Child, '97; Treadwell, '97).

So far as known, the prototroch in the Mollusca arises in a manner which is strikingly similar to its origin in the annelids. Blochmann's derivation of the velum in *Neritina* from the peculiar granulated tip cells of the lateral arms of the cross is doubtless incomplete, since these cells form only a part of this organ in other mollusks, as in annelids. In *Crepidula* the velum at first consists of a double row of cells, the dorsal ends of which become "indistinguishable from the surrounding cells." "The

median portion of the first row," says Conklin, "arises from the cells which lie just beyond the ventral end of the apical plate. These cells are in all probability  $1b^{1,2,2,1,2}$  and  $1b^{1,2,2,2,2}$  [inserting the correction in Conklin's note, p. 204]. One of these cells is shown dividing in Fig. 71. In Fig. 72 a transverse row is formed, which is plainly the first row of velar cells." The portion of the first velar row lateral to these six cells is evidently derived from the anterior turret cells,  $1a^2$  and  $1b^2$ . The anterior turret cells divide bilaterally in a nearly horizontal plane, and these, with the median cells just mentioned, form a row of cells, extending around the anterior half of the egg, connecting the tip cells of the lateral arms. The tip cells of the lateral arms divide, forming a transverse row of four cells, which forms a further continuation of the first row of velar cells as far as the undivided posterior trochoblasts and tip cells.

Regarding the second row, Conklin says: "It is probable that the mid-ventral portion of the second velar row,  $V^2$ , is derived from the cell which I have identified provisionally as  $2b^{2,2}$ , and which lies just beyond the median cells of the first row (Figs. 56, 69, and 70). I have not been able to determine whether any part of the second row arises by subdivision of the cells of the first; if not, this row may include a few cells of the third quartette ( $3a^{1,1,1}$  and  $3b^{1,1,1}$ , Fig. 56) at the points opposite the anterior turrets. . . . Thus the preoral velum is composed of a few cells of the first quartette, many of the second, and possibly a few of the third."

In *Planorbis* the cells composing the prototroch are few in number and are arranged in a double row. The products of the division of the tip cell of the anterior arm of the cross go to form, as in *Crepidula*, a part of the upper row of cells. The tip cell divides, as far as I can determine, but once, and the two daughter-cells become pushed apart by the cell  $1b^{1,2,1,1}$ , which forms the median cell of the upper row. These cells extend to the anterior trochoblasts on either side, but, in later stages, they may sometimes be separated from them by cells which wedge in from below. The anterior trochoblasts, which originally lay, the one above the other, became shifted by the anterior rotation of the upper pole of the egg, so that they come to



lie at nearly the same horizontal level, the originally upper cell lying in front. The tip cells of the lateral arms lie immediately behind the anterior trochoblasts, but do not, as I formerly supposed, form a part of the prototroch, but enter into the formation of the head vesicle. They mark, in fact, the transition between these two structures, and might be considered as greatly enlarged velar cells. The chief differences between the first row of velar cells in *Planorbis* and *Crepidula* are that in the former the lateral tip cells become greatly enlarged and do not divide, and the anterior tip cell divides but once, the daughter-cells becoming pushed apart by one of the cells of the first quartette.

The lower row of cells in the prototroch is derived from the second quartette. Conspicuous among these are the two large cells lying below the median portion of the upper row. They are symmetrically placed on either side the median line. The two cells originally lying above these,  $2b^{1.2.1}$  and  $2b^{2.1.1}$ , have been forced aside by the forward rotation of the upper pole, so that the tip cells,  $2b^{1.1.1}$  and  $2b^{1.1.2}$ , come to lie next to the cells  $2b^{1.2.2}$  and  $2b^{2.1.2}$ . The cells of the lower row at the sides are small, and are added to the prototroch at a later stage, when the number of cells in the regions from which they arose was so great that it would be very difficult to trace their lineage.

### *The Shell Gland and the Foot.*

The shell gland makes its appearance some time after the closure of the blastopore. A very early stage in the development of this structure is shown in Pl. XXI, Fig. 51. It is located a short distance behind the tip of the posterior arm of the cross, in a region which is formed from the cells of the second quartette. It is derived, doubtless, from derivatives of  $2d^{1.2}$  and  $2d^{2.1}$ . It forms a tolerably deep invagination, the cavity of which becomes almost entirely obliterated.

The foot arises as a protuberance behind the mouth. The two halves are separated by a row of clear cells extending backward some distance from the definitive mouth — a fact which may or may not indicate a double origin of this organ. A very

similar group of cells is seen in the middle portion of the foot in *Crepidula* (Conklin, '97, p. 143). Both Lillie and Conklin derive the foot from cells of the second quartette. It seems probable that, in *Planorbis*, cells from the third quartette also enter into its formation. The cells immediately behind the blastopore are derived from the third quartette, and it is not unlikely that the median portion of the anterior end of the foot is derived from some of these cells.

### *The Larval Kidney.*

The larval kidney in *Planorbis* has the form of a V-shaped tube, situated on either side of the body behind the head. At the junction of the two arms is a large perforated cell, the so-called giant cell, the lumen of which communicates with the canals of the arms. The inner arm is directed toward the head; it is formed of a row of perforated cells, and the lumen is ciliated and provided with a ciliated opening near the end. The outer arm is directed downward and is provided with an opening to the exterior. The movements of the cilia lining the arm of the larval kidney may easily be seen in the living embryo.

The larval or head kidney of the pulmonates has been observed several times by the older writers on the embryology of these forms (Stiebel, Ganin, Gegenbaur, Stepanoff), and it has been mistaken for the rudiment of the nervous system, and also for the oesophagus. The giant cell discovered by Bütschli rather strangely had escaped the notice of Fol, who has given an otherwise quite accurate description of this organ.

The development of the larval kidney of pulmonates has been studied by Fol, Rabl, and Wolfson, all of whom came to very different conclusions regarding its origin. According to Fol, the larval kidney in *Planorbis* makes its first appearance as a small ectodermal pouch. "La poche s'approfondit dans la direction du dos, de telle façon qu'elle finirait par rencontrer celle du côté opposé sur la ligne dorsale. Mais la croissance dans ce sens s'arrête de bonne heure, l'organe se recourbe à peu près à angle droit et s'allonge maintenant dans la direction de la bouche." The larval kidney, therefore, according to Fol,



is entirely of ectodermal origin. In a paper on the development of the fresh-water pulmonates, Rabl ('75) described this organ, which he mistook for a part of the nervous system, as arising in a somewhat similar way to that described by Fol.

According to Wolfson's account of the larval kidney in *Lymnaea*, a large velar cell on either side of the embryo is pushed inward, becomes perforated by a canal, and gives rise to a hollow outgrowth which is directed anteriorly and opens by a ciliated mouth. Wolfson holds, in opposition to Fol, that the Urniere are unicellular organs, basing his opinion on the study of a large number of sections.

The account of the origin of the larval kidney given in Rabl's later paper, "*Ueber die Entwicklung der Tellerschnecke*" ('79), differs radically from the foregoing descriptions. According to Rabl's later account, the origin of the larval kidneys can be traced back to a large cell lying in the mesoblastic bands. These large cells, according to Rabl, result from the first division of the mesoblastic teloblasts, though it is more probable they arose from the second (see p. 407). By the teloblastic budding of the two middle cells, two rows of smaller cells are produced which carry forward the large cells,  $v_1$ ,  $v_2$ , at their ends. These large cells also bud off small cells anteriorly, like the teloblasts. Each mesoblastic band comes thus to consist of a large cell with a row of smaller cells in front, followed by another large cell with a similar row in front of it. The cells  $v_1$  and  $v_2$  become perforated by a canal and come to be elongated and bent, forming the giant cell of the larval kidney. Then some of the cells lying in front and behind the giant cells also become perforated and form the anterior and lower arms. The larval kidneys, therefore, according to Rabl, are multicellular organs and entirely mesodermic in origin. The teloblastic budding of the protoblasts of the larval kidneys is a fact of much interest. It recalls the behavior of the nephroblasts described by Whitman ('78) in *Clepsine*, only in *Planorbis* the two teloblastic series are placed end to end, instead of side by side. Such a difference might easily be produced by a variation in the direction of one of the divisions of the teloblasts.

In view of the contradictory accounts of the origin of the

head kidneys, and the important theoretic bearing of the subject, I was led to attempt to verify, if possible, Rabl's description of the development of these organs. I have not followed the subject in detail, but have carried it far enough to satisfy myself of the essential correctness of Rabl's results. The large cells,  $v_1$ ,  $v_2$ , of Rabl are very conspicuous elements of the mesoblastic bands, especially in later stages, as they increase considerably in size. They may frequently be seen perforated by a canal; and one case was found in which the canal did not extend entirely through the cell, but was narrowed to a point near the posterior side. The shape of the canal would seem to indicate that it arose by a sort of invagination at the anterior end of the cell. It may be, however, that the perforation arises, as has been shown in other cases, by the coalescence of a series of intracellular vacuoles. The mesodermic origin of the giant cell of the head kidney is a matter about which, I believe, there cannot be any doubt. Whether a portion of the external canal is ectodermic in origin, as Erlanger ('92) found in *Bythinia*, is uncertain. The principal portion of the structure, however, undoubtedly arises from the mesoderm.

There can be little doubt, when we study the structure and development of the larval kidneys of the pulmonate gasteropods, that these organs represent true nephridia. As the definitive renal organs of the gasteropods are regarded as nephridia also, there occur in the pulmonates two pairs of these organs. Larval kidneys similar to those of the pulmonates have been observed in the embryos of *Oncidium* and some lamelli-branchs. There are sac-like mesodermic larval kidneys in *Paludina* and *Bythinia*, which may be the homologues of the above structures. Whether or not the two pairs of renal organs in *Nautilus* represent nephridial structures, it is quite probable that the existence of the two pairs of nephridia should be regarded as typical for the Mollusca. Whether this indicates that the molluscan body is composed of two segments, is a question which need not here be discussed.











*Gastrulation; Fate of the Blastopore.*

The beginning of invagination may be observed when the egg contains about 175 cells. The egg at this time is a hollow blastula with comparatively thin walls, especially on the upper side. The apical pole at this time has been pushed forward through an angle of about  $45^{\circ}$ , and the rotation continues during the process of gastrulation. For some time before invagination begins the egg flattens and the lower pole loses its convexity. The invagination is first seen in the center of the vegetal pole; the four central cells and the cells of the fifth quartette first sink in, forming a small, round concavity; as this pit becomes deeper, the cells of the fourth quartette are involved in the process. All of the yolk-laden cells become invaginated, and the stomatoblasts may be seen at the edges of the depression. Doubt has been expressed as to whether the cells called entodermic and ectodermic in the early cleavage of mollusks really prove to be so by their history. In *Planorbis*, at least, the edge of the blastopore marks quite sharply the boundary between the protoplasmic and yolk-laden cells. The invaginated area is at first nearly circular in outline, but, as the depression deepens, it assumes an elongated form; and, finally, the mouth of the gastrula becomes reduced to a narrow, slit-like orifice. The length of the elongated blastopore becomes reduced by closure from behind. The anterior end of the blastopore is a comparatively fixed point, and is situated just behind a pair of small cells of the second quartette. Pl. XX, Fig. 46, shows a stage in which the blastopore is reduced to a minute slit lying between two cells. Another preparation showed these two cells in contact, so that the blastopore in this species may be said to close, though it is only for a brief period. The oesophageal invagination occurs very soon after the blastopore closes, *and at exactly the same place*. Its orifice is small and nearly circular, and becomes surrounded by cilia. It gradually deepens and gives rise to a diverticulum on the ventral side, which becomes the pouch of the radula.

The gastrulation in *Planorbis* is purely embolic. The cells of the ectoderm do not slip over the edges of the entomeres, as

in many other gasteropods, even in the least degree. In the species of *Planorbis* studied by Rabl the blastopore is said to become the mouth. Whether this difference is merely a specific one, or whether Rabl failed to observe the blastopore during the short time it is closed, is uncertain.

## PART II. GENERAL CONSIDERATIONS.

### *Reversal of Cleavage and Reversed Asymmetry.*

The fact that in certain gasteropods, with sinistral or reversed shells, the cleavage is also reversed, was first pointed out by Mr. Crampton ('94). Crampton studied the cleavage of two closely related genera of fresh-water pulmonates, *Physa* and *Lymnaea*, the former of which has a sinistral shell, while in the latter the shell is of the normal or dextral type. The cleavage of *Physa* was found to agree, point for point, up to the stage at which the primary mesoblast is formed, with that of *Lymnaea*; but with this exception, that the direction of every cleavage is reversed. The reversal was observed in the cleavage which led to the four-cell stage, and was shown to give rise to a different position of the cross furrow in the two forms. Crampton also points out that *Planorbis*, according to Rabl's paper, affords another instance of reversed cleavage. The possibility of a causal connection between reversed cleavage and a reversal of the shell was pointed out, but further discussion of the subject was not attempted. Haddon's figure of the eight-cell stage of *Janthina* ('82) apparently shows, as Crampton observes, that the cleavage of this form is reversed. This would form the only exception to the rule that the cleavage is dextiotropic in all unreversed forms. It seems not unlikely, however, that Haddon's figure is misleading on this point.

The cleavage of another species of *Physa*, *P. fontinalis*, was found to be reversed by Wierzejski, and Brooks ('79) figures a four-cell stage of *Planorbis parvus*, which, according to his statement concerning the origin of the two upper cells, affords another instance of reversal of cleavage. There are, therefore, two species of *Physa* and three of *Planorbis* in which the cleav-

age is known to be reversed.<sup>1</sup> Planorbis is not usually described, however, as having a sinistral shell; in fact, the shell may be markedly dextral. In the celebrated series of Planorbis shells found in the deposits at Stannheim there is every gradation between shells which are coiled in one plane and shells which are as markedly dextral as those of Littorina or Paludina. Many recent species, *P. albus*, *complanatus*, and *nitidus*, for example, have a shell with a more or less decided dextral coil. Nevertheless Planorbis is a reversed form, whatever the direction of the coil of its shell may be. The anal and genital orifices and the opening of the mantle cavity lie on the left instead of the right side. This is the essential point; the direction of the coil of the shell is a secondary matter. The shell of Planorbis belongs to the type which Lang calls "pseudo-dextral," and has no necessary connection with the essential features of the asymmetry of the animal.

The fact that in five cases a reversed asymmetry of the animal is associated with a reversal of cleavage, while in all dextral forms whose cleavage is known with any degree of accuracy the cleavage is dextiotropic or unreversed, certainly affords a strong presumption in favor of the view that there is some causal relation between the nature of the asymmetry of the body and the type of cleavage of the egg. And there are facts which render this conclusion more or less probable *a priori*. Conklin found that the beginning of asymmetry in Crepidula could be traced back to the cleavage of a single entodermic cell. The time and direction of the cleavage of this cell were found to give the initial bending of the entodermic area in a direction which determined the direction of the coil of the embryo. If we suppose a reversal of cleavage to occur in Crepidula, leading to a corresponding division on the other side of the body, it is not improbable that there would result a reversal of the asymmetry of the animal.

An interesting case in relation to this problem is afforded by the "inverse embryos" of *Ascaris*, described by Zur Strassen ('96). In the normal embryos of *Ascaris* certain cells are

<sup>1</sup> I have recently found reversed cleavage in another sinistral gasteropod, *Ancylus rivularis* Say. (See the *American Naturalist*, November, 1899.)

arranged in an asymmetrical but perfectly definite manner; but Zur Strassen found that in exceptional cases—in one egg out of about forty—this asymmetry was reversed; that is, the arrangement of cells, normally occurring on one side of the embryo, was found on the opposite side, one embryo being the mirrored image of the other. As the adult *Ascaris* is, in certain respects, asymmetrical, Zur Strassen was led to ascertain the proportion of reversed specimens in this form. It was found that, out of 125 individuals, four were reversed. Reversed adults occur, therefore, in about the same proportion as reversed eggs. As reversed eggs were seen, even in advanced stages of development, in an apparently normal condition, Zur Strassen concludes that they develop into reversed adults. We have here a reversal of cleavage occurring as an exceptional variation in eggs of the same species and probably giving rise to a reversal of some features of the structure of the adult form.

In *Planorbis* I have been unable to trace the origin of asymmetry to the cleavage of a single entodermic cell, as Conklin did in *Crepidula*. Immediately before gastrulation begins the entoderm is composed of a nearly circular patch of small entomeres, which are quite numerous (over 30), and of nearly equal size. I am quite certain that, before invagination, the entoderm gives no hint of the direction of the coil of the adult animal, nor does it manifest any appreciable asymmetry until a long time after the gastrula stage. In the gastrula shown in Pl. XX, Fig. 46, the bilateral symmetry is almost perfect. This gastrula contains several hundred cells; yet, if one carefully examines the figure, which is an exact camera drawing showing the outline of every cell, it will be found that for nearly every cell on one side of the body a corresponding cell can be found on the other side. The only deviation from bilateral symmetry that could be observed in this gastrula was a slight torsion in the cells of the head vesicle. The torsion can be observed in most gastrulae, but whether it has any connection with the final asymmetry of the animal could not be ascertained. Attention has been called to the fact that the arms of the cross exhibit a slight twist in a laeotropic direction, and that the direction of the twist is doubtless connected with the reversed cleavage of



this form. This twist is slight, and can no longer be detected when the cross comes to be mainly resolved into two isolated patches of small cells. It is possible that the torsion of the cross is never really lost, and that it is in some way connected with the beginning of the reversed asymmetry of the animal. If this were true, the reversed asymmetry of the adult would be shown to be connected with the reversal of cleavage of the egg. The egg, however, passes through stages of development in which it exhibits a well-nigh perfect bilateral symmetry, so that it seems scarcely possible to connect directly these two phenomena. There must, however, be some structural basis for the asymmetry of the adult in the stages which exhibit such marked bilateral symmetry. The effects of reversed cleavage may be seen in the asymmetrical arrangement of certain cells forming the cross in the first stages of gastrulation. It does not seem improbable that this asymmetry persists in stages in which it can no longer be observed, and that it forms the structural basis of the reversed asymmetry of the adult.

*The Relation between Reversed Cleavage and the Direction of the First Cleavage Plane.*

If we compare the four-cell stages of the eggs of *Lymnaea* and *Planorbis* in respect to the relation of the first cleavage plane to the median axis of the future animal, the fact will become manifest that, in the two cases, this median axis is cut by the first cleavage plane at a different angle. In *Planorbis* and *Physa* the first cleavage plane makes with the median axis a negative angle of about  $45^\circ$ ; while in *Lymnaea* it cuts this axis so as to form with it a positive angle of  $45^\circ$ . It is obvious that, while in both cases the first cleavage plane is oblique to the median axis of the future animal, the first cleavage plane in the sinistral form is at right angles to this plane in the dextral form. As the second cleavage plane is always at right angles to the first, is it not reasonable to suppose that, in the sinistral gasteropods, the first cleavage furrow corresponds with the second in the other forms? Has there not been, in the reversed forms, simply a reversal of the order in which the first two

cleavage planes make their appearance? And is not this the circumstance that determines the different direction of spiral cleavage in the reversed gasteropods?

It has been discovered by Conklin that the first cleavage in *Crepidula* is prospectively spiral and dextrotropic, as indicated by the rotation of the nuclei in the two-cell stage from left to right. I have not convinced myself that there is an opposite rotation of the nuclei in the two-cell stage of *Planorbis*, but the second cleavage is clearly dextrotropic even in its first stages. There is doubtless some structural basis for the different character of the second cleavage in *Planorbis* in the two-cell stage. The second cleavage of *Crepidula* is laeotropic, and that of *Planorbis* dextrotropic; and it seems probable that this difference is connected with the different relations of the first cleavage to the future longitudinal axis of the animal. If we suppose that in *Crepidula* a preformed longitudinal axis is cut obliquely by the first cleavage plane, it may help us to account for the rotation of the nuclei in the two-cell stage, and consequently the laeotropic second cleavage. As this axis in the sinistral forms would be cut at the opposite angle, we may have, in this circumstance, an explanation of the different direction of the spiral cleavage that is manifested in the second, if not somehow in the first division of the ovum. Certain it is that, if the longitudinal axis of the embryo is in any sense preformed in the egg, it is cut at planes approximately at right angles to each other in the dextral and sinistral forms. An oblique cleavage in relation to the bilateral organization of the egg might cause a certain amount of torsion that would manifest itself either at the end of that division or at the beginning of the next. Given two eggs in which this bilateral organization is cut at opposite angles by the first cleavage plane, it is probable that the torsion in the two cases would take place in opposite directions.

#### *General Considerations on Spiral Cleavage.*

If we glance over the literature on spiral cleavage, we shall find that, in different forms, the first cleavage is said to stand in different relations to the future longitudinal axis of the ani-



mal. In most cases the first cleavage plane has been found to be oblique to this axis, and in many forms the median axis is found to lie approximately midway between the first two cleavage planes (Neritina, Physa, Planorbis, Clepsine, Discocelis). In some instances, however, the first cleavage plane is said to be transverse to the median axis of the embryo (Nereis, Umbrella, Teredo). The instances in which the first cleavage plane is said to be oblique are by far the most numerous, and it will be well to devote some attention to the purported exceptions to this rule, with a view to determine whether these exceptions are not more apparent than real. The first cleavage furrow, in advanced stages of cleavage, comes to follow a very crooked path, and the upper portion may run in a quite different direction from the lower. Since the entomeres are usually of large size, the portion of the first cleavage furrow lying between these cells has generally been taken to indicate the direction of the first plane of cleavage, while the upper part of this furrow lying between the ectomeres has been disregarded. It is this circumstance, as will appear, that gives rise to the different accounts of the axial relations of the first cleavage plane. *In the ectodermic portion of the egg the axial relations of the first two cleavage planes are remarkably constant in all forms with spiral cleavage.* The different quartettes of ectomeres have exactly the same relative arrangement in annelids, mollusks, and polyclades; and cells of the same origin in these forms have almost identical axial relations. In both annelids and mollusks certain cells become arranged in the form of a cross, the arms of which have very constant relations to the embryonic axes. The arms of the cross in mollusks, which are always made up of cells of similar origin, are always anterior, posterior, right and left. In the annelids the cross is mainly formed of cells corresponding to those lying between the arms of the molluscan cross, and the arms lie at an angle of about  $45^\circ$  with the median axis of the embryo. As the upper portions of the first two cleavage furrows pass between cells of corresponding origin in all these forms, they maintain almost as constant axial relations as the arms of the cross themselves. *It may be said that the longitudinal axis typically bisects the angle between the upper*

*portions of the first two cleavage furrows.* As Conklin observes, "no exception is known, either among mollusks or annelids, to the rule that the second and fourth quartettes lie in the future median and transverse planes, and that the first, third, and fifth quartettes lie midway between these planes. The axial differences, therefore, of the first two cleavage planes, which have been mentioned, are differences merely in the axial relations of the four primary entoderm cells, and do not affect the axial relations of the other cells of the ovum, which are always the same among annelids and mollusks."

The behavior of the first cleavage furrows in *Crepidula* is very instructive, for it proves that the coincidence of the lower portion of the first cleavage furrow with the transverse axis is not indicative of the axial relations of the first cleavage plane *at the time of its formation*. In *Crepidula*, before the formation of the fourth quartette, the two parts of the first cleavage furrow lie approximately in the same plane. Now the longitudinal axis of the future embryo is definitely marked out at this period by the direction of the anterior and posterior arms of the cross. The first and second cleavage planes at this stage are oblique to these arms of the cross, *and hence to the future longitudinal axis of the embryo*. Thus, as far as can be ascertained, the *original* direction of the first cleavage plane is oblique; subsequent shiftings of the cleavage furrows are of no concern. It is to be noted that it is the upper parts of the first two furrows that retain very nearly their original direction in relation to the median axis, while the axial relations of the lower portions change. The shifting of the lower portions of the first cleavage furrows in no wise alters the fact that the original direction of the first two cleavage planes is oblique. The transverse direction finally taken by the lower portion of the first cleavage furrow in *Crepidula* is, therefore, due to the fact that it has become shifted in relation to the future median axis of the embryo.

In *Nereis*, according to Wilson, the first cleavage plane is transverse, and the second coincides, approximately, with the future median plane. It is evident, as Mead has pointed out ('97, p. 301), that Wilson uses only the portion of the first











cleavage plane lying between the entomeres as the basis of orientation. In *Amphitrite*, according to Mead, "if the second cleavage furrow is followed around the whole egg, its course is found to be an irregular zigzag, but its general direction is at a considerable angle to the sagittal plane." In both *Nereis* and *Amphitrite* the portions of the first two cleavage furrows lying between the ectomeres have essentially the same axial relations. It is only in the portions of these furrows lying between the entomeres that the axial relations vary to any marked degree, and it is probable that in *Nereis*, as in *Crepidula*, the transverse direction of the lower portion of the first cleavage furrow is due to the shifting of the entomeres in relation to the median axis.

It is very probable that if the lower portion of the first cleavage furrow were not shifted in relation to the upper, the whole cleavage plane would be, in every case, oblique to the future median axis of the embryo. In fact, it may be said that, *when- ever the lower portion of the first cleavage furrow has a direction different from that of the upper portion, its direction has been altered in relation to a part of this furrow which lies at a remarkably constant angle to the future median axis of the embryo; this is consequently tantamount to becoming shifted in relation to the median axis itself.* In such cases the lower portion of the first cleavage furrow can no longer be considered indicative of the direction of the first cleavage plane.

It is apparently, therefore, a universal characteristic of forms with spiral cleavage that the first cleavage plane is oblique to the future longitudinal axis of the embryo. As far as is known, the first cleavage plane, in forms with normal or unreversed cleavage, always makes a positive angle with the future median axis, while it makes a negative angle with this axis in forms whose cleavage is of the reversed or sinistral type. These facts, I believe, are not devoid of significance in relation to the general theory of spiral cleavage. In the previous section the suggestion was made that the difference in the direction of the first spiral cleavages in *Crepidula* and *Planorbis* might be due to the circumstance that the first cleavage plane cuts a pre-formed longitudinal axis in one form at a positive, and in the

other at a negative, angle. We are naturally led from this supposition to the view that spiral cleavage, in general, may be due to the oblique direction of the first cleavage in relation to a preformed longitudinal axis of the ovum. The possibility is open, on the other hand, that the direction of the first cleavage is not predetermined in the ovum, and that the median axis is determined only during cleavage. The evidence, however, is apparently against such a view, although it is still far from complete. The fact that the isolated blastomeres of the gastropod egg exhibit the phenomenon of "partial development" (Crampton, '96) in a marked degree affords a very strong reason for regarding the median axis of the embryo as determined at the time of the first division. It does not necessarily follow that this conclusion is to be extended to all forms with determinate spiral cleavage; yet Crampton's experiments may rightly be held, I think, as affording no little support to this generalization.

If the first division were always of a spiral character, as it is in *Crepidula*, the second cleavage would, as a consequence, be also more or less oblique, and the spiral character of the succeeding cleavages may be regarded as simply a consequence of Sach's law, that the direction of every cell division tends to be at right angles to the preceding division. The second division in forms with determinate spiral cleavage is, I believe, always a spiral one. In *Planorbis*, as in *Physa* (Crampton, '94), *Limax* (Kofoid, '95), *Crepidula* (Conklin, '97), and *Amphitrite* (Mead, '97), the spiral character of this cleavage is manifested by the inclination of the spindles before a very marked constriction of the cytoplasm takes place, and cannot, therefore, be regarded as a consequence of the shifting of the blastomeres. It seems not improbable that the spiral character of the second cleavage is a consequence of a spiral tendency of the first cleavage — that the agencies that cause the rotation of the nuclei and spheres in *Crepidula* after the first division are present in other eggs also, although they produce no visible effect until the second cleavage. In both annelids and mollusks, spiral cleavage is soon superseded by cleavage of the bilateral type, and there appears no reason to doubt that bilateral cleavage in

these forms is correlated with the bilaterality of the future animal. May not the delayed appearance of bilaterality be due to the circumstance that the oblique direction of the first cleavage started the divisions in a spiral direction, which is only overcome later by the tendency to bilateral cleavage?

It is worthy of note, in this connection, that in many eggs the first cleavage plane is oblique to the axis of elongation. This is the case, according to Conklin, in *Urosalpinx*, and, according to Fol ('75), the first cleavage furrow in *Cymbulia* is oblique to the line connecting the two attraction spheres. Among the Rotifera, Tessin ('86) found that in *Eosphora* the first cleavage furrow is oblique to the long axis of the egg and, therefore, to the median axis of the animal. In *Callidina*, according to Zelinka ('91), the first cleavage plane is oblique to the long axis of the egg, but by a shifting of the cells it comes, finally, to be transverse. And in *Asplanchna*, Jennings found that the first cleavage amphiaser "lies at first somewhat oblique to the longitudinal axis of the egg, but before cleavage takes place the spindle swings into coincidence with it." The nucleus in the larger of the two cells subsequently rotates to the right, so that a line joining the two nuclei would cut the first cleavage plane at an oblique angle. The possibility suggests itself that the rotation of this nucleus may be due to the same circumstances that caused the oblique direction of spindle before cleavage. The behavior of the egg of *Asplanchna* would seem to indicate that it tends to divide obliquely, as in *Callidina* and *Eosphora*, but that this tendency is overcome by the tendency to divide at right angles to its longest diameter, and only manifests itself at the beginning and at the end of cleavage. The first cleavage of *Asplanchna* recalls that of *Crepidula* in that the phenomenon of nuclear rotation is manifested after the completion of division. It is quite evident that among these rotifers other factors besides the shape of the egg influence the direction of the first cleavage. According to Hertwig's law, it would be expected that the first cleavage plane would uniformly lie at right angles to the long axis of the egg. The fact that the first cleavage is oblique to this axis in *Callidina* and *Eosphora*, and manifests a tendency to obliquity in

*Asplanchna*, points to the conclusion that the egg possesses a certain degree of cytoplasmic organization, which tends to determine the cleavage in a certain direction, even in opposition to Hertwig's law. And this naturally leads one to suspect that the first cleavage plane may be predetermined in eggs like those of most mollusks and annelids in which no means of orienting it are apparent.

The observations of Blochmann ('82) on *Neritina* show, if correct, that in this form it is very probable that the longitudinal axis of the embryo is determined before the first division. Blochmann found that in the two-cell stage the granules which later go into the tip cells of the lateral arms of the cross were aggregated into two small patches on the two sides of the egg. If these groups of granules existed, as seems very probable, before the first cleavage, it would show that the axial relations of the embryo were determined in the undivided egg. "Aus dem Umstand," says Blochmann, "dass die hellen Körnchen schon auf dem Stadium der Zweitheilung vorhanden sind (Fig. 39), kann man wohl folgern, dass die beiden Anhäufungen auch schon in dem eben in die Furchung eingehenden Ei vorhanden waren, und dass sie die Enden eines Durchmessers einnahmen, also eine Achse bestimmten." And Blochmann adds, in a footnote: "Bei unbefruchteten Eiern habe ich manchmal nach dem Austritt der Richtungsbläschen um den animalen Pol eine Anhäufung von solchen hellen Körnchen beobachten können (Fig. 23 bis 26), wage jedoch vor der Hand nicht zu entscheiden, ob dieselben hierher zu beziehen sind." It is unfortunate that Blochmann's observations on this latter point were not more decisive. Could the chief axes of the ovum be determined before fertilization, it would manifestly exclude the possibility that the entrance path of the spermatozoön determines the longitudinal axis of the future embryo. If in any eggs with determinate spiral cleavage the direction of the first cleavage plane and, consequently, the median axis of the future embryo is determined only after fertilization, as is claimed by Mead in the case of *Chaetopterus*, it may still be true that the spiral character of the first cleavages is determined by a bilateral organization of the egg substance, which is developed between



the period of fertilization and the occurrence of the first division of the ovum. The direction of the first cleavage plane and the direction of the spiral tendency of the first cleavage (whether to the right or the left) are two different things and may well be determined at different times. Roux has discovered that, in the frog's egg, the plane of inclination of the egg axis, as well as the direction of the first cleavage, is determined by the entrance path of the spermatozoön. Although in *Rana esculenta* the axis of the eggs is oblique before fertilization, it was found by Roux that the inclination of the primitive egg axis stands in no constant relation to inclination of the axis of the fertilized egg, which is determined by the entrance of the sperm. There is thus in the frog's egg a redistribution of some of the egg substances after fertilization and before the first cleavage, with reference to the future plane of symmetry, if there be not a differentiation also of the cytoplasm. That the period between fertilization and cleavage is a period of active differentiation in the eggs of many forms is a conclusion to which many facts point. Further investigations will have to be made, however, before it can with certainty be determined whether, in eggs with spiral cleavage, the chief axes are established before the first division of the ovum by any sort of differentiation of the egg substance.

In eggs with bilateral cleavage it is the rule that the first cleavage plane and the median axis of the embryo coincide. There are several exceptions to this rule, however, but they occur in forms in which the cleavage is not of a highly determinate type, as is shown by the variations in the cleavage of different eggs of the same form. Where the cleavage is definite and determinate, the cell divisions occurring with regularity and precision, with little individual variation in the eggs of the same species, the direction of the first cleavage plane, in eggs with bilateral cleavage, appears to mark accurately in almost every case the direction of the median axis of the embryo. This is notably the case in the cleavage of the ctenophores (Metschnikoff, Agassiz, Chun). In the cephalopods bilateral cleavage is again beautifully illustrated (Kölliker, Bobretzky, Vialleton, Ussow, '81; Watasé, '91); though subject to some

individual variation (Watasé, '91), the early cleavage is quite definite, but soon the divisions become too irregular to follow in detail. In the tunicates we find bilateral cleavage of a most conspicuous and determinate type, the median axis of the embryo, as in the preceding forms, being marked out by the first cleavage furrow (Seeliger, van Beneden and Julin, Castle, '96). Among the vertebrates there are no cases of that regularity and determinateness of cleavage which characterize the early development of many invertebrates. The cleavage is quite regular in some forms until a certain period, after which the divisions follow no apparent order. In most forms whose cleavage has been carefully studied there has been found considerable variation in the cleavage of different eggs of the same species. To the extent, however, that the cleavage of the eggs of vertebrates follows any definite plan, it may be said to belong to the bilateral type. The first cleavage plane often coincides with the median axis of the embryo, though in some forms it may apparently form any angle with this axis. In *Amphioxus*, although the form of cleavage is exceedingly variable, exhibiting cases of radial, spiral, and bilateral divisions in different eggs, there is a predominant tendency to bilaterality in the early cleavage (Wilson, '93), and the first cleavage marks the future longitudinal axis. The cleavage of fishes, after the first few divisions, usually becomes quite irregular. In *Batrachus*, according to Miss Clapp, the first cleavage plane may form a considerable angle with the median axis of the embryo. The early cleavage of this form is, nevertheless, quite markedly bilateral, though, as Miss Clapp informs me, it is subject to considerable individual variation, and the plan of cleavage becomes very irregular in later stages. Morgan found, also, in *Fundulus* that, while the cleavage up to a certain stage was of the bilateral type, the first cleavage bears no constant relation to embryonic axes. While in the frog and some other *Amphibia* the first cleavage plane and the median axis of the embryo commonly coincide, the first cleavage plane in *Diemyctylus* (Jordan) and *Triton* (Hertwig) is usually transverse to this axis. The axial relations of the first cleavage in case of the frog, however, are known to be largely influenced by gravity



(Pflüger), and in *Diemyctylus*, Jordan found that the first cleavage plane often deviated considerably ( $45-50^{\circ}$ ) from the transverse axis.

In *Amblystoma*, Jordan and Eyclescheimer found that the first furrow became so irregular that they considered it improbable that it should ever come to separate, exactly, the right and left halves of the embryo; and the same conclusion may well be drawn from the cleavage of many other vertebrates. Where, in eggs with bilateral cleavage, the first cleavage plane forms an oblique angle with the median axis, this angle varies in different eggs, *i.e.*, the first cleavage plane does not stand at a *constant* oblique angle to the median axis. When the first cleavage plane has *constant* axial relations, it is either median or, more rarely, transverse (*Polychoerus*, Gardiner, '95). Determinate bilateral cleavage may readily be conceived to occur in which the first cleavage furrow stands at a certain constant oblique angle to the median axis, the second at right angles to the first, and only the third or fourth meridional cleavage furrow coinciding with the median plane of the embryo. But the course of bilateral cleavage does not run in this manner. Where, as in the toadfish, the first cleavage plane may form a considerable angle with the median axis, and the cleavages, up to a certain period, are symmetrical in relation to this plane, it is obvious that, unless an extensive shifting of the blastomeres occurs, the cleavage cannot be of the determinate type; cells of corresponding lineage cannot have the same fate in different eggs. In cases like this the form of cleavage can hardly be held to express a bilateral organization of the egg substance and cannot have much morphological significance. Even in eggs devoid of bilateral organization the cleavage might form, according to Sach's law, a more or less definite pattern, whose form would be largely dependent on the amount of yolk in the egg, extrinsic conditions, etc.; or the organization of the egg may be such as to exert no influence on the early cleavage. But in cases where bilateral cleavage is *determinate*, where cells of the same lineage always have the same fate in different eggs, the first cleavage plane apparently always coincides with either the median or the transverse axis of the embryo. Where the first

cleavage plane does not coincide with either of these axes, bilateral cleavage, when it occurs, is of the indeterminate type. The converse proposition also appears to hold good, *viz.*, where the cleavage is determinate and the first cleavage plane coincides with the median or transverse axis, the cleavage is of the bilateral type. If these rules have exceptions, they are sufficiently general not to be devoid of significance. The cleavage of the ovum is influenced by a large number of factors, both internal and external. Of these factors the degree of organization of the egg and the direction of the first cleavage plane with reference to the planes of symmetry of this organization play, I believe, an important part.

*Reversal of Cleavage and Cell Homologies.*

Detailed study of the early developmental stages of annelids and mollusks has brought to light numerous and striking points of resemblance between the cleavage of various members of these groups; and the cleavage of the polyclades, as shown by Lang's work in *Discocelis*, is so similar to that of the above forms that it may properly be considered as belonging to the same general type. Professor Wilson, in his paper on the "Cell Lineage of *Nereis*," has called attention to the close resemblances of the cleavage of the annelid *Nereis* to that of the gasteropods *Crepidula* and *Neritina* and the polyclade *Discocelis* as follows: "Up to a late stage in the spiral period (twenty-eight cells) every individual blastomere and every cell division is represented by a corresponding blastomere and a corresponding cell division in the embryo of the polyclade, and in that of the gasteropod. In all three the first two cleavages and the upper and lower cross furrows have the same relations. In all, three groups of four micromeres each are successively separated from the macromeres,—the first group in a right-handed spiral, the second in a left-handed spiral, and the third in a right-handed spiral, like the first. The micromeres of the second and third groups alternate with one another so as to form an outer belt of eight cells that surrounds the four primary micromeres."











Professor Wilson also pointed out that in all three groups the first cleavage of the first and third quartettes occurs in the same direction, and that the cleavage of the gasteropods and the annelid is characterized by an additional point of agreement, in that the primary mesoblast cell,  $4d$ , is given off in both cases in a left-handed spiral, from the left posterior macromere  $D$ .

It was found later by Lillie ('95) that the cleavage of *Unio*, one of the lamellibranchs, agrees with that of the gasteropods and annelids, not only in the features mentioned, but in several other points of undoubted morphological significance; and Conklin has since added many other striking resemblances which characterize the cleavage of annelids and gasteropods. The discovery made by Conklin that the turret cells in *Crepidula* have the same origin, position, and, in the main, the same fate as the trochoblasts of the annelids, and that the prototroch, in both groups, is completed by certain cells of the second quartette, affords one of the most notable points of similarity in the cleavage of these forms.

Resemblances such as these would seem to indicate, as Conklin strenuously insists, that cleavage has a much greater morphological significance than has usually been assigned to it. Yet, while recognizing all these wonderful similarities between the cleavage of different classes of animals, does it follow that the form of cleavage has any really fundamental connection with the process of development? While it is true that there are numerous cases in which cells of corresponding origin and position have the same fate in widely separated groups, there are other instances in which cells of the same origin have a very different fate in forms which are much more closely allied. (Compare the fate of the larval mesoblast cell in *Unio* with that of the same cell in *Planorbis*.) And there are also cases in which it has been found that cells which have the same fate have a quite different origin, even in the same class of animals; instance the origin of the secondary mesoblast in *Crepidula* and *Planorbis*. It is hard to reconcile these facts on the view that the process of cleavage has any fundamental connection with the homology of organs. Neither can the similarity in the cleavage of different groups be accounted for by attributing it

to extrinsic mechanical conditions. The resemblances between the cleavage of mollusks and annelids are too numerous and too close to be explained in this manner, or even, as I believe, by the principle of "parallel precocious segregation." But while similarity of cleavage in different groups, provided it is long continued and close, may be held to indicate genetic affinity, the converse of the proposition, that differences in the form of cleavage imply lack of relationship, does not always follow. It is well known that the form of cleavage may vary even in eggs of the same species. The summer and winter eggs of daphnids have entirely different forms of cleavage, yet both develop into the same kind of embryos. In many vertebrates the particular manner in which the egg is divided appears to be a matter of little moment as regards its future development. In Renilla, Wilson found that the early cleavage of the egg presented great variations which were without any apparent influence on the end result.

It is obvious that no general rule can be drawn regarding the phyletic significance of cleavage. In some groups cleavage has, doubtless, a high degree of "systematic worth"; in others it may have very little. Similarly, as a mark of affinity between the different groups of animals, as in the case of gasteropods and annelids, cleavage may be a character of considerable value; or again, as in the case of the gasteropods and cephalopods, its evidence may be of little weight. It seems probable that similarities of cleavage should be regarded as an *incidental* and not a *necessary* expression of genetic affinity. Whether or not the relationship between different classes of animals expresses itself in the early cleavage of the ovum may depend largely upon external conditions, or upon the amount of yolk in the egg, or, perhaps, upon the degree of cytoplasmic differentiation that has been reached before cleavage begins. It is not my purpose, however, to attempt to discuss what may be the reason for the varying morphological significance of cleavage forms in different groups of animals. The fact I would emphasize is that mere cell genealogy stands in no *necessary* relation to the genealogy of organs. This conclusion, which is supported by a variety of considerations, receives a strong confirmation — if

not a demonstration — through the facts of reversal of cleavage. It can readily be seen that, by virtue of reversed cleavage, the relative positions of certain cells become different from those they would occupy if the cleavage were of the normal or unreversed type. For example, the position of the cells of the third quartette in each quadrant is, in the dextral forms, to the right, and, in the reversed forms, to the left of the cells of the second quartette. Similarly, the trochoblasts lie to the right of the apical cells in the dextral forms, and to the left of these cells in the sinistral forms. In the dextral forms the cells in the two anterior quadrants of the third quartette are  $3a$  and  $3b$ , and in the posterior quadrants  $3c$  and  $3d$ . In the reversed forms  $3b$  and  $3c$  are anterior, and  $3a$  and  $3d$  posterior. In the dextral forms the trochoblasts are  $1a^2$ ,  $1b^2$ , anterior, and  $1c^2$ ,  $1d^2$ , posterior; while in the reversed forms the anterior trochoblasts are  $1b^2$ ,  $1c^2$ , the posterior,  $1a^2$ ,  $1d^2$ . In both reversed and unreversed forms the corresponding arms of the cross are derived from the same quadrants.

The anterior trochoblasts in both *Crepidula* and *Planorbis* go to form the prototroch, and the posterior ones go into the head vesicle. The cells which have a similar position in the two forms have the same fate, although they have a different origin. The cells which go into the prototroch in *Crepidula* are  $1a^2$  and  $1b^2$ , while in *Planorbis* the cells which have this fate are  $1b^2$  and  $1c^2$ . Conversely, cells of the same cell origin have different fates, *viz.*,  $1a^2$  goes into the prototroch in *Crepidula*, while in *Planorbis* it forms a part of the head vesicle. Although the cell  $1b^2$  goes into the prototroch in both forms, it forms a part of the *right* side of this structure in *Crepidula* and a part of the *left* side in *Planorbis*. It certainly appears that, in a certain sense, the fate of a cell is a function of its position. It has been remarked by Driesch that, if the blastomeres of an egg could be shifted about at will, their development would take place in accordance with their relative positions. While in reversed cleavage nature has performed an experiment for us in the shifting of blastomeres, and while the results show that the fate of the cells is in accordance with their position, and not their genealogy, the experiment differs considerably from

the hypothetical shifting process of Driesch. The blastomeres are not shifted about promiscuously, but cells occupying similar positions to those in the unreversed eggs contain cytoplasm derived approximately from the same portion of the ovum; and it may be for this reason, and not on account of the mere fact of position, that they come to have the same destiny. Corresponding portions of the egg cytoplasm develop along similar lines, whose direction appears to be independent of the precise form of cleavage, even when the cleavage is of a highly determinate type. The direction of every cell division may be reversed up to a late period of cleavage without altering the fate of the cells having the same position in the egg.

Reversal of cleavage, like the pressure experiments of Driesch on echinoderm eggs, and of Wilson on the eggs of *Nereis*, shows that the immediate causes of the differentiation of cells lie, not in the nucleus, but in the cytoplasm. While certain cells have the same position and fate in reversed and unreversed eggs, the nuclei of these cells have entirely different lines of descent; and, conversely, nuclei having the same origin come to lie in entirely different portions of the embryo. Thus, in perfectly normal development, the fate of cells appears to be entirely independent of the origin of their nuclei. How this fact can be reconciled with the view that the differentiation of blastomeres is mainly the result of qualitative nuclear divisions, I cannot understand, unless we suppose that there is some complex mechanism for the proper sorting of nuclear material to provide for the contingency of reversed cleavage.



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## EXPLANATION OF PLATE XVII.

- FIG. 1. Egg mass of *Planorbis trivolvis*.  
FIG. 2. Undivided egg.  
FIG. 3. 2-cell stage, showing the second cleavage spindles and the torsion of the dividing cells.  
FIG. 4. 4-cell stage viewed from the side, showing the laeotropic division of *B*.  
FIG. 5. The same egg seen from the upper pole, showing the laeotropic inclination of the spindles.  
FIG. 6. 8-cell stage seen from the side.  
FIG. 7. The same egg seen from the animal pole.  
FIG. 8. Apical view of the 24-cell stage.  
FIG. 9. Same egg seen from the side.  
FIG. 10. Same egg seen from the vegetal pole, showing the division of *D* which produces the primary mesoblast cell *D*<sup>4</sup> or *M*.  
FIG. 11. View of the lower pole of the 33-cell stage, showing the large mesoblast cell lying partly in the cleavage cavity.  
FIG. 12. Lateral view of the same egg, showing the cleavage of the lower tier of the second quartette.













## EXPLANATION OF PLATE XVIII.

FIG. 13. Apical view of an egg in the 49-cell stage.

FIG. 14. Lower side of same egg, showing the mesoblastic pole cells,  $M^1$  and  $M^2$ , lying partly in the cleavage cavity.

FIGS. 15, 16, and 17. Lateral views of the same egg.

FIG. 18. Egg of about 54 cells seen from the vegetal pole. The cells of the fourth quartette are seen in process of division.

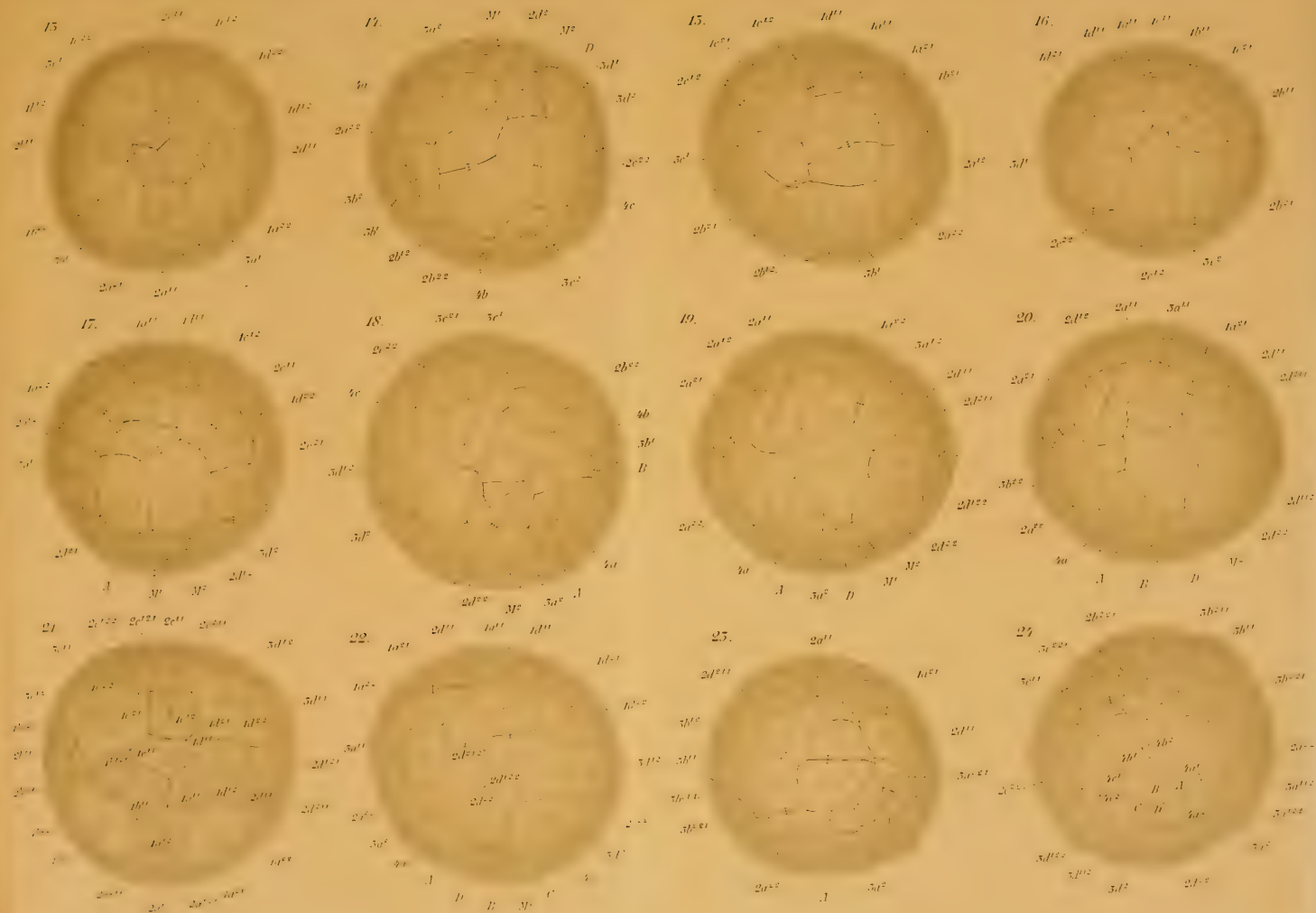
FIG. 19. Lateral view of same egg, showing dextrotropic cleavage of  $2a^{1,2,1}$ . By an error the same figure is repeated in Fig. 20.

FIG. 21. Apical view of an egg of about 64 cells, showing a division of two of the basal cells of the cross.

FIG. 22. View of posterior side of the same egg. The portion of the mesoblastic pole cells,  $M^1$  and  $M^2$ , exposed at the surface of the egg, has become considerably reduced.

FIG. 23. Lateral view of an egg of about 80 cells, showing the division of the upper pair of cells of the third quartette in the  $\alpha$  quadrant.

FIG. 24. Vegetal pole of an egg of about the same stage. The mesoblastic pole cells have entirely disappeared from the surface.









## EXPLANATION OF PLATE XIX.

FIG. 25. Apical view of an egg of 104 cells. The trochoblasts have increased in size, become clearer, and have apparently compressed the arms of the cross which stand out in greater contrast to the cells between them than in the earlier stages of development.

FIGS. 26, 27, 28, and 29. Lateral views of the same egg. It will be seen that the cells of the second quartette in the *b* quadrant have not kept pace with the divisions of those of the other three quadrants. In Fig. 29,  $3a^{1.2}$  has divided, while its fellow,  $3a^{1.1}$ , remains entire; the corresponding divisions have both occurred in the *d* quadrant.

FIG. 30. Lower pole of same egg. The cells of the fourth quartette have undergone a second cleavage.

FIG. 31. Apical pole of an egg of 130 cells. The tip cells of the cross have enlarged and become clear, the arms show a marked laeotropic twist, and the splitting of the arms is begun by a transverse division of one of the cells of the anterior arm. The apical pole has begun to rotate forward, and the posterior trochoblasts have become slightly larger than the others.

FIG. 32. Left side of same egg.

FIG. 33. Anterior side of same egg. The third quartette in the *b* quadrant consists of a double row of four cells. In the *c* quadrant the divisions of the third quartette are somewhat more advanced.  $3c^{2.2.1}$  has just divided, while the corresponding cell,  $3c^{2.1.1}$ , is entire.  $3c^{1.2.2}$  has divided, and its fellow,  $3c^{1.1.2}$ , is undergoing division. The upper cells of the second quartette are beginning to become clear and to take on the character of the neighboring trochoblasts.

FIG. 34. Right side of same egg; the forward rotation of the apical pole may be seen here as in Fig. 32.

FIG. 35. Posterior side of same egg.

FIG. 36. Vegetal pole of same egg. A fifth quartette has been formed, and the cells of the fourth quartette in the *b* quadrant have undergone a division at right angles to the previous one; the corresponding cells in the *a* and *c* quadrants remain undivided.













## EXPLANATION OF PLATE XX.

FIG. 37. Apical view of an egg of about 150 cells.  $2b^{1.1}$  is in process of division, although this change had taken place in the earlier stage shown in Fig. 31. A longitudinal splitting has taken place at the base of the lateral arms of the cross.

FIG. 38. Vegetal pole of the same egg. The lower side is flattened and slightly depressed in the center. In the  $b$  quadrant two of the secondary mesoblast cells have lost connection with the surface, and two others,  $3b^{2.2.1.2}$  and  $3b^{2.1.1.2}$ , have still a small portion of their surface visible at the outside of the egg.

FIG. 39. Anterior view of an egg of about the same stage as the preceding. The tip cells of the anterior arm of the cross have become clear, and are being pushed by each other by the forward rotation of the apical pole. The lower cells of the second quartette are somewhat dislocated by the same process.

FIG. 40. Apical view of a later stage, showing the two upper cells of the second quartette of the anterior side of the egg now lying side by side.

FIG. 41. Vegetal pole of an egg of about the same stage. The depression of the entoderm is deeper than in Fig. 38. A division has apparently occurred in  $2b^{2.2.1}$  and in  $1b^{2.2.1}$ . The middle cells of the anterior quadrant of the second quartette,  $2b^{2.1.1}$ ,  $2b^{2.1.2}$ ,  $2b^{1.2.1}$ , and  $2b^{1.2.2}$ , have been dislocated by the rotation of the apical pole so that they lie in a transverse line. The secondary mesoblast cells no longer appear at the surface of the egg. The stomatoblasts in the  $a$ ,  $b$ , and  $c$  quadrants still remain undivided, while the corresponding cell of the  $d$  quadrant is crowded away from the entomeres by the cells of the third quartette, which come to meet in the middle line (*cf.* Figs. 30, 36, 38, and 41).

FIG. 42. Apical pole of a later stage. The anterior rotation of the apical pole is increased, and the cells of the middle of the cross are beginning to enlarge and become clear.

FIG. 43. Right side of same egg.

FIG. 44. Lower pole of same egg; mouth of gastrula reduced to a slit.

FIG. 45. Posterior side of a gastrula, showing the large head vesicle.

FIG. 46. Ventral side of the same gastrula, showing the blastopore reduced to a minute slit lying between a pair of oblong cells. Prototroch shown by a band of clear cells in front of the blastopore. Rudiments of the cerebral ganglia shown by two patches of dark cells separated by a median band of large, clear cells, the apical plate *A.P.*

FIG. 47. A later stage, showing the prototroch, apical plate, *A.P.*, blastopore, *bl.*, and the rudiment of the right cerebral ganglion. The cell boundaries shown in the figures in this plate are not diagrammatic, but represent accurately the outlines of every cell. In eggs stained with silver nitrate these boundaries are as clear as shown in the figures.

FIG. 48. View of anterior portion of a gastrula, showing the cell  $1b^{1.2.1.1}$  pushed forward until it comes in contact with the prototroch.















## EXPLANATION OF PLATE XXI.

FIG. 49. Mesoblastic pole cells, showing the small cells budded off at the anterior end.

FIG. 50. Optical section of an egg of about the stage shown in Fig. 41. *P.M.*, primary mesoblast; *S.M.*, secondary mesoblast.

FIG. 51. Posterior side of a young embryo, showing the large head vesicle, *h.v.*, and the beginning of the shell gland, *s.g.*

FIG. 52. Optical section of a slightly later stage. *M.*, mouth; *s.g.*, shell gland; *c.g.*, rudiment of cerebral ganglion which is proliferating cells; *p.*, prototroch; *e.c.*, entodermic cells that have become gorged with albuminous matter; *mes.*, cells of the mesoblastic band; *g.c.*, giant cell that has become partially perforated.

FIG. 53. Surface view of the same stage. *M.*, mouth; *p.*, prototroch; *c.g.*, rudiment of cerebral ganglion. The patch of clear cells around the mouth gradually narrows posteriorly until it becomes a narrow band of clear cells separating the two halves of the foot.





49



50

SM

PM



52

mes

mu

p

est

st

st



54

st<sup>2</sup>

st<sup>2</sup>

st<sup>2</sup>

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## THE DEVELOPMENT OF THE COXAL GLAND, BRANCHIAL CARTILAGES, AND GENITAL DUCTS OF LIMULUS POLYPHEMUS.

WM. PATTEN AND ANNAH PUTNAM HAZEN.

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## I. INTRODUCTION.

THE work described in the following pages was mainly done, during the year 1895 and 1896, in the Biological Laboratory of Dartmouth College, and was continued through the summer season of 1896 in the Marine Biological Laboratory at Woods Holl.

The problem we had in mind at first was the development of the branchial cartilages, but it was finally deemed advisable to work out, in connection with this problem, the development of the genital ducts and the "coxal gland," or nephridia, as all three of these organs are closely associated with one another during development.

We were in a certain measure prepared for the independent origin of the nephric lobes and nephric duct by the discovery that in the adult kidney, as seen by the aid of injections, a distinct nephric duct is present, which could hardly be anything else than the tube seen by other investigators in the embryos.

This left the development of the nephric lobes to be accounted for in some other way than as a modification of the embryonic duct.

We are indebted to Professor Whitman for the privileges of the Marine Biological Laboratory at Woods Holl and to Dr. William A. Redenbaugh for assistance in working out the course and origin of the nerves found in the region of the nephridial lobes.

## II. METHODS.

The *embryological material* was killed either in Perenyi's fluid, picro-nitric acid, picro-sulphuric acid, corrosive sublimate, or formalin. The most satisfactory results were obtained with Perenyi's fluid. The embryos remained in it overnight. In the morning the much distended membranes could be easily removed, and the eggs placed at once in large quantities of 95% alcohol, which was changed often to prevent the yolk from swelling and cracking. Low grades of alcohol must be avoided in the preliminary stages of hardening.



The embryos were stained *in toto* with Delafield's haematoxylin or with borax carmine, followed by Lyon's blue on the slide. The larvae and young Limuli were treated with Delafield's haematoxylin, and picro-acid fuchsin, or eosin.

The *nephric duct* may be injected by forcing a canula into its external opening at the base of the fifth leg, or it may be ligatured into the end of the duct, just below the shell.

Starch masses, or a thick celloiden mixed with lamp black, were used for injections. After celloiden injections are hardened in 80% alcohol for several days, the connective tissue may be dissected away from the duct, and its course followed without much difficulty. Complete casts of the duct may be obtained by maceration in strong hydrochloric acid; but in all cases the casts which were made showed a good deal of shrinkage. A good starch, asphalt, or celloiden mass will completely fill the duct and penetrate deeply into the nephridial lobes.

### III. CRITICAL REVIEW.

The Nephridia of Limulus were first described by Packard in 1875, who concluded that they were renal organs, comparable with the green glands of Crustacea. In 1880, he compared them with the shell glands of the Entomostraca.

Lankester described them in 1880, and later ('82) discussed their histological structure in detail, comparing them with the coxal glands of scorpions and Mygale. Both Packard and Lankester saw only the lobes and the longitudinal stolon of the adult, and entirely overlooked the large thin-walled duct and its external opening. Packard tried to find a duct by injecting the gland, but failed. In 1895 Tower discovered the opening of the gland in the adult in the interarticular membrane on the posterior side of the base of the fifth leg. It is situated, as he correctly states, on a papilla, readily seen with the naked eye, and surrounded by a dark gray ring.

Gulland ('85) and Kingsley ('85 and '93) gave an incomplete description of the embryonic duct and its relation to the fifth coelomic cavity. They were deceived, however, in mistaking the developing duct and its end sac for the developing nephric

lobes, while the nephric lobes themselves were entirely overlooked. They were further misled by not knowing the existence of the adult duct with its permanent external opening, and other characters, which clearly distinguish it from the nephric lobes.

Kingsley ('93) supposed that the four nephridial lobes of one-half of the body arose from a portion of the coelom of the fifth somite, which had retained its position on the ventral side of the embryo after the other coelomic cavities had emigrated toward the dorsal surface. The fate of the dorsal portion of the fifth somite was not determined, but he states that it persists as a perfectly distinct cavity with epithelial walls on either side of the heart. From its position and from its posterior termination he is inclined to think that this portion of the coelomic epithelium is finally converted into the reproductive organs (p. 200). "For a considerable time," he says, the ventral portion of this coelomic cavity "shows no change worthy of remark." At length, however, it "begins to elongate and to become bent upon itself like the letter *U*, the rounded portion being directed anteriorly." An ingrowth of ectoderm unites with this tube to form its outer opening, situated on the posterior side of the coxa of the fifth leg. He also states that "in the neighborhood of the coxal gland at this stage may be seen numerous lacunae in the mesoderm, which is rapidly assuming the trabecular condition characteristic of the later stages" (p. 203). He was unable to trace any connection between these lacunae and the coelomic cavity, and is strongly of the opinion that none exists. Again he states (p. 204) that there is a "formation of trabeculae of mesodermal tissue which invade the cavity, and, passing from wall to wall of the proximal portion of the organ, tend to subdivide it and give it an anastomosing character." In the older stage he finds a fenestration in the region of the end sac, which is "the beginning of the anastomosing condition of the adult, while the proximal (internal) limb is thrown into a series of four outwardly directed diverticula, which are segmentally arranged and occupy somites 2-5" (p. 205). Kingsley suggested "that the whole organ of the adult is derived from the coelom of somite *V*, and that the apparently metameric lobes figured by

Packard . . . are not the remnants of the Nephridia of the corresponding somites, but are rather the derivatives of the diverticula of the duct" (p. 205). He maintains that, "besides an increase in the size of these lobes, all that is necessary to convert his reconstruction into the 'coxal gland' of the adult are closures of the external opening, more or less complete fusion of the two limbs of the duct, accompanied by an increase in the anastomoses, the result being to convert coelom and duct into the spongy tissue of the adult." Mr. Gulland's account does not differ materially from this, but is not as complete. He speaks of septa, which grow inside the tube and divide it. In one place he found "the tube continuous with connective-tissue spaces, which everywhere surround the gland."

Our own observations have shown that there is no division of the fifth somite into a dorsal and a ventral portion. It is very clear, also, that the nephric duct is not a transformation of the ventral portion of this somite, but a special epithelial outgrowth from it, and that the rest of the somite persists as the end sac only. There are no segmental diverticula of the duct, and no part of the duct is converted into the glandular tissue of the nephric lobes. It remains practically unchanged, except in length and in the number of its convolutions, as the permanent nephric duct of the adult. The nephridial lobes are derived from segmental clusters of cells which arise independently of the duct from the somatic layer of the second, third, fourth, and fifth thoracic somites.

It is obvious that the comparisons and conclusions of our predecessors in this line of work were based on incomplete anatomical and embryological data. We must still wait a more thorough study of the anatomy and development of the green glands, coxal glands, and shell glands of other arthropods before their relations to one another can be satisfactorily determined.

#### IV. FORMATION OF THE MESOBLASTIC SOMITES AND BLOOD SINUSES.

*The Thoracic Somites.*—Soon after the formation of the thoracic appendages, and before the abdominal ones have

appeared, a solid mass of mesoderm cells is seen at the base of each thoracic appendage. Each mass of cells gradually extends in a lateral direction to form a transverse band quite distinct from those on the opposite side of the body. Beneath the mesoderm, and enclosing the yolk, is a thin non-cellular membrane (Pl. XXIV, Figs. 35 and 36), which gradually separates from the central portion of each mesodermic band, but remains continuous with it around its margins. At the same time nuclei appear to migrate from these margins into the membrane. In this way an imperfectly closed sac, the *somite*, is formed with a very thick outer wall, the somatic, and a thin inner one, the splanchnic layer (Pl. XXIV, Figs. 37 and 38).

Each somite now grows rapidly in a lateral direction, making with its mate on the opposite side almost a half circle. The concave side of the posterior thoracic somites is directed backwards, that of the anterior somites forwards.

As the appendages grow in length, a space is formed between the ectoderm, forming the apex of the leg and the thick somatic mesoderm at its base. A few scattered mesoderm cells remain attached to the inner surface of the ectoderm, and they gradually form the walls of a spacious cavity, which later is converted into the blood channel that passes through the center of each leg. We could not certainly determine whether this space is to be regarded as a part of the coelom, as in the abdominal sinuses, or not.

The further history of the thoracic somites, except the fifth, has not been carefully studied.

*The abdominal somites* develop in a different way and are much more clearly defined. They separate from the paired bands of mesoderm formed by the primitive streak as hollow masses, with distinct and continuous walls of nearly uniform thickness. Each abdominal somite is at first quite separate from all the others, and its cavity remains closed for a long time. Figs. 2-8, Pl. XXII, are longitudinal sections showing the first three abdominal somites and a part of the primitive streak in an embryo in which the abdominal appendages are beginning to appear.

In this series the median ends of the somites are thin-walled



tubes; towards their lateral ends their somatic walls are much thickened to form the muscular tissues of the appendages.

The chelarial segment, it will be observed, contains a well-defined abdominal somite, although it is smaller than those in front or behind it. This fact shows conclusively that the chelaria are true appendages, having the same morphological value as the other appendages of the body.

The opercular somite is the largest, the following ones gradually diminish in size from before backwards.

Figs. 8 and 9, Pl. XXII, represent longitudinal sections through an older specimen. They show four fully formed abdominal somites, with a fifth one just separating from the anterior end of the primitive streak.

The somites grow over the surface of the yolk in a postero-lateral direction, till they unite in the median dorsal line. The circular bands of mesoderm thus formed become smaller and smaller in diameter from the opercular segment towards the posterior end of the body.

At an early period the distal ends of all the somites become continuous with a thickened band of proliferating cells that forms a well-defined margin extending round the whole embryonic area (see Patten, '90, p. 373).

The somites of the opercula and gill-bearing segments persist as the well-defined blood sinuses leading from the abdominal appendages to the pericardium.

## V. THE DEVELOPMENT OF THE GENITAL DUCTS.

The genital ducts arise as diverticula from the median ventral side of the opercular somite, and extend towards the median line along the base of the opercular cartilages. They lose (?) their connection with the somites as soon as two or three gill leaves are formed on the first branchial appendage, and then remain in a very rudimentary condition until after the second larval stage. The distal ends of the diverticula finally unite with shallow invaginations of the ectoderm, while their proximal ends unite in a manner not yet determined with the genital organs.

The first traces of the genital ducts were found in embryos having three abdominal somites (Pl. XXII, Figs. 1-8). In this series the sections begin near the median line and extend towards the lateral ends of the somites. Near the median line are a few loose mesoderm cells. Those near the surface of the yolk are undergoing degeneration. Their nuclei contain large granules that stain deep red in borax carmine, and the nuclear membrane seems in some cases to have ruptured.

The lumen of each somite increases in size as one moves away from the median line. At the same time the somatic layer increases rapidly in thickness, till just beneath the appendages it is many cells deep. The splanchnic layer contains only a few isolated nuclei. The lateral ends of the somites have no lumen and consist of mesoderm cells not arranged in distinct layers.

About midway between the median and lateral ends of the opercular somite the genital duct appears as a long diverticulum, extending from the coelom into the somatic layer (Pl. XXII, Fig. 6, *g.d.*). A similar diverticulum, although not as marked, is shown in the first gill in Pl. XXII, Fig. 5. It was not determined whether this was a rudimentary genital duct or the beginning of the branchial cartilage.

In Pl. XXII, Figs. 2 and 3, a body resembling an extra somite is present between the operculum and the first gill. Its median end was hollow, while its lateral extremity was united with the mesoderm of the first gill (Pl. XXII, Figs. 2-4, *SO*<sup>o</sup>). Such a structure was not seen in any other series of sections of this age.

In the next stage five abdominal somites are present. They are shown in Pl. XXII, Figs. 9-20, which represent a series of longitudinal sections beginning near the median line and extending almost to the lateral ends of the somites.

In the opercular and first gill segment the somites have moved towards the yolk, leaving behind at the base of each appendage a *thick ring of mesoderm*, derived from the somatic mesoderm (Pl. XXII, Figs. 12-20, *SO*<sup>1</sup>-*SO*<sup>2</sup>). As the appendage grows in length, a space is formed between its ectoderm and the ring of mesoderm at its base; and in these spaces and around the



somites are found numerous blood corpuscles, but free cells are never found at this stage inside the somites.

The chelarial somite is large and distinct near the median line (Pl. XXII, Fig. 9,  $SO^1$ ), farther away from the median line it becomes constricted and nearly disappears (Pl. XXII, Fig. 11,  $SO^1$ ), and still farther away it again becomes more distinct (Pl. XXII, Figs. 12 and 13,  $SO^1$ ).

The genital duct at this stage is considerably enlarged. It forms a diverticulum on the ventral wall of the opercular

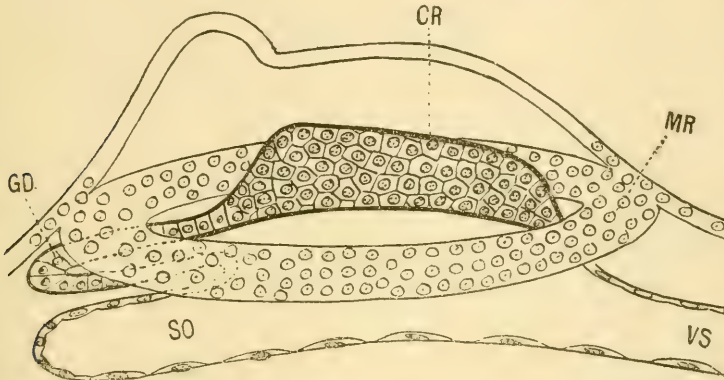


FIG. 1. — A diagram showing the relative positions and shapes of the cartilage, the genital duct, and the somite in the operculum. The somite is a large cavity at the base of the operculum, closed at the median side and extending on the surface of the yolk beyond the outer ectodermic margin of the appendage. On its ventral margin the somite pushes down into the space in the appendage and at the same time bends towards the median line, making a closed diverticulum. The diverticulum lengthens and becomes relatively smaller, and breaking free from the somite it finally becomes the genital duct (*g.d.*). On the ventral side of the diverticulum is a band of cells which form the cartilage rod (*c.r.*). Around the base of the operculum and on the ventral side of the somite is a ring of mesoderm cells. On its median and lateral sides it is continuous with the ectoderm.

somite, with its blind end directed towards the median line. By comparing the different sections of this series it will be seen that the opercular somite is swollen at its median end (Pl. XXII, Fig. 9,  $SO^2$ ), and reduced to a narrow tube on the median side of the point where the genital duct arises from it (Pl. XXII, Figs. 11 and 12,  $SO^2$ ). On the lateral side of this point the somite again enlarges to form a spacious chamber (Pl. XXII, Figs. 16–20,  $SO^2$ ).

The relations of the genital duct, appendage, mesodermic ring, and cartilage to the opercular somite are shown in the accompanying diagrammatic cut (Fig. 1).

In the next series of sections (Pl. XXIII, Figs. 21-24) the genital duct is seen to open into the opercular somite, just median to a transverse plate of mesoderm cells that will ultimately develop into the opercular cartilage.

In a still later stage, where one gill leaf has formed on the posterior surface of the first branchial appendage (Pl. XXIII, Figs. 25-29), the ectodermic portion of the appendage extends a considerable distance beyond the ring of mesoderm around its base. Slender processes extend from the ectodermic cells of one wall to those on the opposite side. In the space beyond the mesoderm, blood corpuscles are frequently found. Pl. XXIII, Fig. 25, represents one of the sections from this series near the median line, showing the genital duct (*g.d.*) directly under the base of the cartilage. The cartilage has now grown out from the ventral wall of the somite in a median direction over the genital duct, and it also extends some distance ventrally into the appendage. Following this series of sections laterally, the somite is next seen at the posterior side of the genital duct (Pl. XXIII, Fig. 26) with a band of muscle cells (*m.*) upon its dorsal wall. The partition between the somite and the duct becomes thinner, until finally the duct and somite unite, as shown in Pl. XXIII, Fig. 27.

In the next stage to be described two or three gill leaves have formed on the first branchial appendage (Pl. XXIII, Figs. 30-32). The operculum is greatly elongated, and its opposite walls are united by numerous outgrowths of the ectoderm. The remnants of the mesodermic ring are still plainly visible at its base. The genital duct is relatively smaller than in the preceding stage and extends in a transverse direction along the dorsal edge of the opercular cartilage. Its proximal end now loses its connection with the opercular somite and remains for some time in a rudimentary condition. In the second larval stage it can be found at the base of the opercular cartilage as a short tube, still unconnected with either the ectoderm or the coelom.

In specimens half an inch long it was well developed and united at its distal end with an infolding of the ectoderm. The manner in which its inner end becomes united with the genital organs has not been determined.

## VI. THE DEVELOPMENT OF THE BRANCHIAL CARTILAGES, MUSCLES, AND NERVES.

The cartilages in the abdominal appendages of *Limulus* first appear as solid outgrowths of the ventral wall of the somites. The following descriptions and drawings produced as proof of this statement are based in the main on what takes place in the operculum. The cartilages in the other abdominal appendages develop somewhat later, but in a very similar manner.

In an embryo of three abdominal segments, as shown in Pl. XXII, Figs. 1-8, where the genital duct is seen as a diverticulum on the median ventral wall of the somite, there is no trace of cartilage in the thick somatic layer of mesoderm. But when five abdominal somites are developed the cartilage can be easily distinguished (Pl. XXII, Figs. 9-20, *cr.*). At this time the somite has moved toward the yolk, leaving a large part of the somatic layer behind, as the ring of mesoderm around the base of the appendage. At the same time a transverse plate of cells is formed subtending the ring. This plate of cells is the "Anlage" of the opercular cartilage. Its ventral edge hangs freely into the cavity of the appendage; its median and lateral ends are continuous with the cells forming the mesodermic ring, while its dorsal edge is continuous with the ventral wall of the somite. The opercular cartilage is therefore derived from the ventral wall of the somite and lies just lateral to the point where the genital duct opens into it. At this time the cartilage cells do not differ in histological appearances from those in the surrounding mesoderm.

The cartilage of the first gill-bearing appendage develops in a similar manner, but more slowly than that in the operculum, as seen in Pl. XXII, Figs. 12-16, *SO*<sup>3</sup>. There are openings in the anterior wall of the first gill somite that appear to afford a normal communication between the coelom and the surrounding blood spaces. The posterior wall of the gill contains a mass of ectoderm cells in active division, evidently preparing for the formation of the gill leaves which appear there at a later period.

The relations of the mesodermic ring, cartilage, genital duct, and somite are shown in cut 1.

In the next stage, where one gill leaf is developed on the first branchial appendage (Pl. XXIII, Figs. 25-29), the opercular cartilage has grown rapidly; its ventral edge projects into the mesodermic ring, but the median and lateral sides are still continuous with it.

In the preceding stage (Pl. XXII, Figs. 15-20) the cartilage extended only from the point of union of the genital duct with the somite to the lateral margin of the mesodermic ring. In this stage (Pl. XXIII, Figs. 25-29) it has grown in a median direction along the ventral wall of the genital duct, so that the two structures are for a short distance completely fused with each other (Pl. XXIII, Figs. 25 and 26, *cr.*).

The cartilage cells now begin to show the features that characterize them so clearly in the later stages; *viz.*: (1) they are larger than the surrounding mesoderm cells and have distinct cell walls; (2) they are arranged in rather regular order; and (3) the protoplasm stains very lightly in borax carmine.

The first gill cartilage shows a very similar condition to that in the operculum.

In the next stage, with three gill leaves on the first branchial appendage (Pl. XXIII, Figs. 30-33), the cartilages form long flat plates that extend some distance beyond the ring of mesoderm into the appendage (Pl. XXIII, Fig. 33). The cartilage of the first gill is attached to the anterior wall of its appendage and extends from there to the somite at its base. A section nearer the lateral line would show a similar attachment of the opercular cartilage to the anterior wall of its appendage. The cartilage cells are of uniform size and have a characteristic appearance and arrangement in rows. The cell walls are sharply outlined against the clear homogeneous protoplasm. The nuclei are large and oval and deeply stained. The cartilage is surrounded by a thick hyaline membrane, which is continuous with the outer wall of the somite.

In the early Trilobite stage (Pl. XXIII, Fig. 34) the cartilages differ but little, except in size, from those in the adult. Each abdominal somite has now become a venous sinus bounded by a thin membrane. The dorsal wall of the sinus is composed of small oval cells, which take a very deep stain. On the ven-



tral side the cells are larger and gradually merge into the cells composing the branchial cartilages.

The ectodermic cells on the dorsal side of the embryo are long and slender and arranged in rows, with their nuclei near their outer ends. At the dorsal extremity of the cartilage there is a point where the boundaries between these ectoderm cells, the cartilage, and muscles merge together, and it is impossible to distinguish between them (Pl. XXIII, Fig. 34, *x*).

A perichondrium is now visible, composed of a layer of spindle-shaped cells, apparently derived from the mesodermic ring, and not from a transformation of the peripheral cartilage cells. The latter, as we have shown, are formed from the mesothelium of the somatic walls.

The distal ends of the branchial cartilages finally fuse with the ectoderm that forms the anterior wall of the appendage. At these points the cartilage and ectoderm are so completely united that it is not possible to distinguish their original boundaries. Thus, an appearance is produced that might easily mislead one into believing that the cartilages were growing as inward proliferations from the ectoderm. But, aside from the fact of their union at these points, there is no evidence that such is the case.

The spaces in the distal ends of the appendages are crossed by fibrous columns arising from the ectodermic walls. At the base of each column are several nuclei, as though the columns were formed by the union of several cells. No mesoderm extends into the appendages beyond the distal ends of the cartilages.

*The Abdominal Muscles.* — Distinct muscles are first seen on the dorsal wall of the somite of the operculum and the first gill in specimens in which one gill leaf is developed (Pl. XXIII, Figs. 25–29, *m*). They increase rapidly and grow into the yolk, forming the dorso-ventral muscles of the adult. There are also stout bands of longitudinal muscles which extend from the anterior end of one somite to the posterior end of the preceding somite. All the mesoderm cells of the mesodermic ring are apparently converted into muscles attached to the base of the abdominal appendages.

*The Branchial Nerves.* — In studying sections of young embryos the mesodermic ring is often found fused with the ectodermic wall of the appendage, so that it is impossible to distinguish between them. In some of these places were found what appeared to be large ganglionic or sensory cells, no doubt derived from the overlying thickenings of the ectoderm. They differed in general appearance and coloring from the surrounding cells, and were most numerous at the posterior margin of the appendages, between the mesodermic ring and the ectoderm. In older specimens nerve cells and fibers were found in corresponding positions (Pl. XXIII, Figs. 25 and 26, *n.*).

In a later stage (Pl. XXIII, Figs. 30 and 31) the nerves are clearly outlined in both the operculum and the first gill. In a still older embryo (Pl. XXIII, Fig. 33), on the posterior side of the first gill, is a proliferation of nerve cells and fibers resembling a sense organ. It represents the same proliferation seen in Pl. XXIII, Figs. 25–27. From it is formed the first gill leaf. The nerve fibers in question are the large ventral nerves that supply the abdominal appendages. They appear to develop, therefore, as fibrous outgrowths (of the central nervous system ?) that receive at various points along their course fibers and cells from the overlying ectoderm, and especially from those points where the gill leaves are about to appear.

## VII. THE NEPHRIC GLAND.

A. *The Development of the Nephridial Lobes.* — The nephridial lobes are formed from loose clusters of cells, situated near the base of each of the six thoracic appendages. The cells show a distinctly segmental arrangement, and are derived from what appears to be the median somatic wall of the mesoblastic somites. The nephridial cells of the first and sixth appendages degenerate and disappear after the second larval stage. Those in the second, third, fourth, and fifth appendages form the nephridial lobes of the adult.

The nephric duct arises in quite a different manner, as a tubular diverticulum of the somatic wall of the fifth thoracic somite; the nephridial cells of this segment do not appear till



long after the duct, and the lobes in the other thoracic segments are clearly differentiated.

Nephridial cells are first seen in the fourth segment at about the time the somite appears. They appear a little later in the third segment, and then in the second, first, sixth, and fifth in the order named.

The nephridial cells may be seen before the abdominal appendages are formed, or before the somites are clearly outlined, on the dorsal wall of the mass of mesoderm at the base of the appendages (Pl. XXIV, Fig. 35, *n.c.*). This mass of mesoderm corresponds to the mesodermic ring of the abdominal appendages, and later forms the somatic wall of the somite. The splanchnic layer is at this period represented by a thin non-nucleate membrane next the yolk.

The nephridial cells may be recognized by their larger size, their sharp nuclear stain, and by their lighter colored, transparent protoplasm. They are best developed near the center of the mesodermic mass at the base of the appendage, and from that point they gradually merge in all directions into the surrounding mesoderm.

In the next stage (Pl. XXIV, Fig. 36) the nephridial cells are larger and more numerous, and some of the oldest ones contain a few small granules.

In the next stage (Pl. XXIV, Fig. 37) they have increased greatly in size and numbers, and are very conspicuous, owing to the deep blue color they take when treated with Lyon's blue. The oldest cells have developed slender pseudopodia-like processes.

The membrane over the yolk contains distinct cells, forming the splanchnic layer of the somites, and the latter are as distinctly differentiated as at any time in their development. They do not at any time form closed sacs like the abdominal somites.

The section shown in Pl. XXIV, Fig. 38, passes through the posterior margin of the fourth leg of the same embryo, and shows the reduction in size and grade of development of the cells on the periphery of the future nephric lobes.

Pl. XXIV, Fig. 39, represents a section through the middle of the fourth nephric lobe in a still older stage. A few cells

appear to be leaving the central mass and moving along the walls of the somite towards the dorsal side of the egg. Some of them may possibly give rise to the nephric cells, which at a much later period are found around the pericardium.

The above description applies to the nephridial cells of the fourth leg; but a similar series of changes has also taken place in the chelicerae and the second and third legs. At a corresponding place in the fifth leg the duct of the nephridia has been developing. The true nephridial cells do not appear in that appendage until later. A longitudinal section (Pl. XXIV, Fig. 40), a little to one side of the median line, shows these bunches of developing nephridial cells. In the fifth leg the section passes through the end sac of the nephridial duct. In the sixth leg there is a cavity similar to the end sac, but it is not in the plane of the section.

The nephridial cells of the fourth and fifth leg are shown on a much larger scale ( $\times 400$ ), in a longitudinal section, in Pl. XXIV, Fig. 41. Here the cells are crowded with large spherical granules that stain an intense blue in Lyon's blue. The cell walls are often very faint and easily overlooked. A few of these cells are seen in the fifth leg on the ventral wall of the end sac. The walls of all the thoracic somites have broken down and disappeared, except those of the fifth somite, which is now more clearly outlined as the end sac of the nephric duct (*e.s.*).

In a longitudinal section of an embryo, just before the Trilobite stage (Pl. XXV, Fig. 43), the segmental arrangement of the nephric cells is still clearly shown. The cells are relatively larger than before, and the granules are breaking up into smaller ones (Pl. XXIV, Fig. 41). During the Trilobite stage a marked change occurs in the character of the nephridial cells. The oldest ones have elongated and become irregularly cylindrical, the coarse granules have disappeared, and the finely granular protoplasm has collected around the periphery. The nucleus has also taken up its position just inside the cell wall (Pl. XXV, Fig. 45, *n.c.* and *g.n.c.*). These hollow elongated cells then unite end to end, forming a loose network of branching tubules (Pl. XXV, Fig. 47, *n.c.*).











These changes begin first in the center of each segmental mass of nephric cells, so that for a time each set of tubules is unconnected with the tubules in the adjacent segments.

Some nephric cells are still present like those described in the earlier stages (Pl. XXVIII, Fig. 80, *c* and *d*). On the periphery of the lobes are small granular cells that stain deeply in Lyon's blue, and which appear to be blood corpuscles (*s.r.c.*).

The formation of nephric tubules now begins to extend forward and backwards, forming at first a few slender chains of cells uniting the ventral ends of the nephric lobes with one another. As these connecting cells become canalated, the branching tubules in each lobe are united into one system. The various kinds of cells seen in the nephridial lobes are shown in Pl. XXVIII, Figs. 74, 75, 77, and 79.

In a crab about one inch long a cross-section in the region of the fifth leg (Pl. XXV, Fig. 48) shows that the nephridial lobes now consist of several distinct layers or strata, composed of cells in different stages of development. The end sac (*e.s.*) on the median side opens through numerous tubules into smaller ones, which again break up in the loose tissue of the gland. The tubules in the region of the end sac are large, and open with such wide mouths into the end sac that it is not possible to determine just where the sac ends and the tubular tissue begins. The layer of large tubules nearest the end sac is lined with pavement cells filled with coarse granules. These tubules are evidently formed from the hollow cell chains of the previous stage by the multiplication of the nuclei and the breaking up of the peripheral protoplasm into separate cells. Thus, the intracellular chain of vacuoles is changed into the intercellular lumen of a duct lined by many flattened cells.

The next layer is composed of chains of cells with finely granular protoplasm and conspicuous nuclei. Most of the cells are hollow, and united end to end to form a network of intracellular tubules like those seen in the second larval stage (Pl. XXV, Fig. 47).

The outer layer or cortex of the lobe consists of many small cells with very conspicuous nuclei. Among them are some

enormous cells filled with refractive spherules. One large spherule usually occupies the center, surrounded by many smaller ones, so numerous as to completely hide the nucleus (Pl. XXVIII, Fig. 77).

The division of the nephric lobes into concentric layers is not a sharp one, still it is clearly evident on careful examinations of the sections with moderately high powers. Pl. XXV, Fig. 48, was drawn on too small a scale to show these different layers well. There can be no doubt that the nephric lobes from now onward increase in size exogenously, and that the stratified appearance of the lobes in section is due to a succession of different stages of development that begins with indifferent mesoderm cells on the periphery and ends with the fully formed intracellular tubules in the center of the lobes.

The nephridial lobes of the second, third, fourth, and sixth leg resemble those in the fifth but the stratification of the layers in the latter is more clearly marked. Cross-sections of the stolons uniting these lobes also show very clearly the concentric strata.

The difficulty of following the history of the nephridial cells is much increased by the granules, which come and go, and which change the appearance of the nephridial cells so much that it is hard to recognize their various phases. They appear to accumulate in the cells until the canalization of the latter and their union to form a system of connecting tubules afford the necessary means for their discharge into the end sac, and from there to the exterior. It is certain that the granules begin to diminish in numbers about the time the nephric duct acquires an opening to the exterior.

*B! Structure of the Nephric Gland in the Adult.*— In young Limuli about two or three inches long the nephridial cells form compact masses of tissue easily distinguished from the surrounding organs. The cells at the base of the first and sixth appendages have disappeared; those at the base of the four remaining appendages form the four permanent lobes of the kidney.

In the adult the lateral surface of each lobe, except the first (Pl. XXVIII, Fig. 83), is flattened and lobulated, with a roughly

slipper-shaped outline. This is the growing surface of the lobe, and contains just beneath the outer layer the finest tubules and capillaries. The median side of the lobe is somewhat wedge-shaped, the coarsest ducts being nearest the apex of the wedge. At the ventral ends of the lobes the wedge-shaped surfaces gradually widen, as the coarser tubules diverge to meet those forming the stolon uniting the four lobes. The network of coarse longitudinal ducts of the stolon empty into the end sac situated in the middle of the fifth lobe (*e.s.*), and from there the secretions pass to the exterior through the long nephric duct.

The size and outline of the different lobes, especially the first one, vary a good deal in different individuals.

The nephric gland lies deeply imbedded in the muscles around the base of the legs, and can be readily recognized in the fresh condition by its brick-red color. In some specimens the surface is a pale yellow, or is mottled with red patches. The inside of the lobes, however, was always brick red. Each nephridial lobe had two ear-like lobules attached to its median ventral end near the stolon. On the first lobe they were large, massed one above the other, and entirely covering the collecting tubes. On the remaining lobes they were much smaller, and were connected with them by a slender stalk.

A short distance from where the pedal arteries leave the circum-oral ring a large blood vessel arises which extends laterally along the posterior ventral margin of each nephric lobe. It passes directly through the median portion of each lobe to supply the muscles and other tissue beyond. Before entering the nephric lobes many branches are given off, the anterior ones supplying the nephric lobes, the posterior ones the adjacent muscles. These blood vessels form a rich mass of capillaries round the nephric tubules.

*Nerves.* — Two sets of nerves pass close to or through the nephric glands. The haemal, or integumentary, nerves of the third, fourth, and fifth thoracic neuromeres pass through the stolon, and between the nephric lobes, to the sides of the carapace, without apparently giving off any branches to the gland (Pl. XXVIII, Fig. 83, *int.n.*, and Pl. XXVI, Fig. 49, *n.*).

Eight smaller nerves arise from the roots of the pedal nerves and supply the coxal muscles at the base of the coxite. There are two of these nerves to each lobe, one on either side (Pl. XXVIII, Fig. 83, *ex.n.*<sup>1-8</sup>). The third, fourth, and seventh nerves pass directly through the stolon; the second, fifth, sixth, and eighth nerves pass over its dorsal, and the first over its ventral, side.

No branches could be found running from these nerves into the lobes, although sections show the presence of numerous fine nerve bundles ramifying through the lobes in all directions.

In sections of the adult gland one may distinguish five concentric layers, each layer containing nephridial tissue in different stages of development. Beginning at the center of the lobe, we have in order: (1) large collecting tubes (Pl. XXVI, Fig. 54); (2) small clear-walled tubules, Fig. 53; (3) tubes lined with granular cells, Fig. 52; (4) chains of vacuolated cells, Fig. 51; (5) large granular cells, Fig. 50.

The large granular cells are very numerous on the ventral and dorsal sides of the nephric lobes (Pl. XXVI, Fig. 49, *g.c.*). Under a higher power two or three nuclei are sometimes seen in a single cell. In borax carmine and Lyon's blue, or in Delafield's haematoxylin and eosin, the cell wall takes a dark stain and appears as a fine thread among the unstained granules. Wedged in between them were occasional bunches of from five to ten or more small dark-colored cells, probably blood corpuscles (Pl. XXVI, Fig. 50, *b.g.c.*).

Small bundles of nerve fibers are abundant in the granular tissue, especially on the median dorsal side of the nephridial lobe (Pl. XXVI, Fig. 50, *n.*). In fresh specimens this tissue has a dull orange color and resembles adipose tissue. A layer of loose connective tissue forms an indistinct boundary between the cells just described and the true nephridial cells.

The latter form the layer marked *h.c.* in Pl. XXVI, Fig. 49. It consists of small cells with large dark nuclei (Pl. XXVI, Fig. 51). The innermost cells are vacuolated, and have fine granular protoplasm on the periphery, and some have united end to end to form delicate intracellular tubules like those seen in



the early Trilobite and second larval stages. These cells and tubules represent the peripheral terminations of the system of tubules leading into the end sac. This layer nearly surrounds the lobe. It is thickest at its apex, becomes thinner on the median ventral side, and disappears entirely on the median dorsal side, where the longitudinal collecting tubes unite the lobe with one another. Within this layer is one formed of tubules, lined with large granular cells, as shown in Pl. XXVI, Fig. 49, *g.t.*, and Pl. XXVI, Fig. 52. They are surrounded by a loose connective tissue, containing nuclei larger than those in the granular cells. There are two kinds of nuclei in the walls of the tubules, one small, dark, and homogeneous, the others larger, and showing clearly the chromatin granules.

In the next layer (Pl. XXVI, Fig. 49, *t.p.*, and Pl. XXVI, Fig. 53) the cells have lost their granules and have flattened out to form a thin endothelial lining to the tubules. The tubules are large, and really form a meshwork of spaces separated by vacuolated connective tissue. Blood channels, containing large granular blood corpuscles, are abundant in the connective tissue surrounding the tubules. The large collecting tubules are best developed in the center of the lobe and on the dorsal surface at their median ends. The endothelium of these tubes (Pl. XXVI, Fig. 54) stains more deeply, and is vertically striated on its surface farthest from the lumen of the tubes, next the very distinct basement membrane. The tubes are widely separated by a spongy connective tissue, richly supplied with blood vessels.

#### VIII. THE NEPHRIC DUCT.

*A. The Development of the Nephric Duct.*—The nephric duct develops as an evagination of the somatic mesoderm of the fifth leg. The duct cells appear before the nephridial cells of that segment, and before the boundaries of the somite are clearly defined, as an oval plate of columnar cells, easily recognized by their large size and clear protoplasm (Pl. XXVI, Figs. 55-57). At the edges of the plate they pass gradually into the undifferentiated mesoderm that covers the yolk. Beneath the

plate is a noncellular membrane forming the boundary of the yolk. On the median side of the center of the plate is a shallow outfolding (Pl. XXVI, Fig. 56, *end.*) that marks the beginning of the tubular portion of the duct.

In the next stage this outgrowth (Pl. XXVII, Figs. 59-64) has formed a short tube, with its solid distal end growing towards the median line and meeting the ectoderm at the base of the fifth leg; the margins of the original plate now form the funnel-shaped opening (nephrostom ?) into the underlying space.

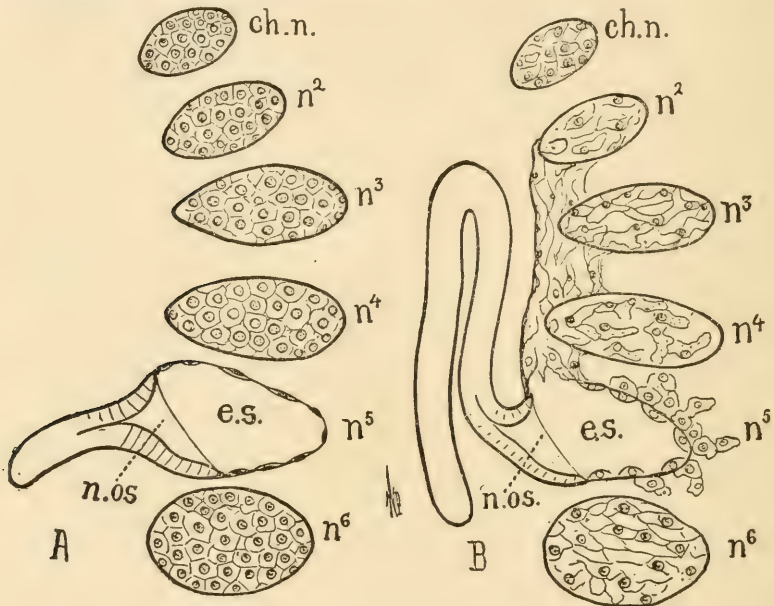


FIG. 2. — Diagrams representing two stages in the development of the nephridial lobes and the duct. They represent the left halves of the nephridia seen from the neural side. Clusters of the nephridial cells are seen in the chelicera, the 2d, 3d, and 4th legs. The end sac and nephridial duct are in the 5th leg. At this time the nephridial cells are large and granular. The end sac is a closed cavity with a few granular nephridial cells appearing on its ventral surface.

The lips of the funnel have gradually united with the membrane over the yolk, and at the same time nuclei migrate into the membrane. Thus, a closed sac is formed which we have called the *end sac*. The entire ventral wall is formed by the nephric plate, which represents the somatic layer of the fifth somite. The end sac apparently represents the coelomic cavity of that somite, and the dorsal wall is formed from the splanchnic layer of the somite.



A proliferation of the ectoderm on the posterior median side of the fifth leg is seen in Pl. XXVII, Fig. 63, *ect.p.*, marking the beginning of the ectodermic infolding, which in the following stage unites with the distal end of the nephric duct.

In the next stage (Pl. XXVII, Figs. 65-70) the distal end of the duct has united with the ectodermic infolding (Pl. XXVII, Fig. 71). The nephric plate is still visible as the flaring mouth of the duct (Pl. XXVII, Figs. 65 and 66), the lateral lip being much longer than the median one.

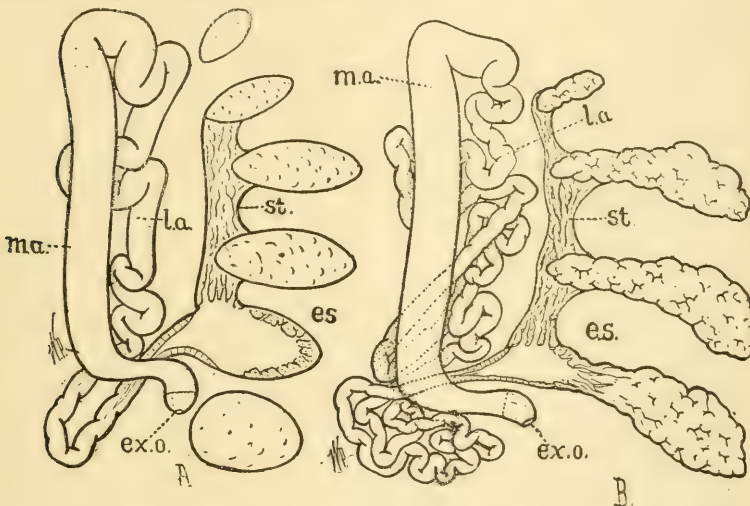


FIG. 3 A. — Diagram of the 2d larval stage, showing nephridial cells in the chelicera, the 2d, 3d, 4th, 5th, and 6th legs. The end sac is at the base of the 5th leg, surrounded by nephridial cells. The duct extends forward to the 2d leg, and backward to the 6th. The proximal arm is much coiled, the distal arm is nearly straight.

FIG. 3 B. — Diagram representing the adult condition. The median ends of the lobes have grown forward and backward and united to form the stolon. This portion of the nephridia is composed of large collecting tubules, which carry the excreta to the end sac in the 4th lobe; from there they pass into the nephridial duct.

The relative size and positions of these parts are shown in a little earlier stage in Pl. XXVI, Fig. 58.

In the next stage the duct elongates very rapidly, and as each end is fixed a  $\Pi$ -shaped tube is formed with the loop reaching forward to the middle of the fourth leg.

The ectodermic portion of the duct is very short (Pl. XXVII, Fig. 73), and may be readily distinguished by its small deeply stained nuclei and by its delicate internal lining of chitin.

During and after the Trilobite stage the lateral arm of the duct becomes convoluted and a second loop is formed near its proximal end, directed backwards and medianly, and lying dorsal to the median arm of the first loop (cuts 3 A. and 3 B.). The median arm of the first loop becomes considerably dilated, and apparently acts as a reservoir for the secretions of the gland. It remains a straight tube throughout life. The anterior end of the lateral arm (*l.a.*) is smaller and somewhat convoluted, the foldings increasing in number and extent towards the posterior loop.

The structure of the nephric duct during the Trilobite stage is shown in Pl. XXV, Fig. 45. The cells lining the duct now have no distinct cell walls, although the walls are easily seen in the preceding and in the following stages.

The anterior loop of the duct now extends as far forward as the second leg. The following parts may be distinguished (cut 3 A.); *viz.*: (*a*) the short ectodermic portion; (*b*) the dilated median arm of the anterior loop; (*c*) the slightly coiled lateral arm of the anterior loop; (*d*) the much coiled posterior loop; (*e*) the end funnel; (*f*) the end sac.

*B. The End Sac.*—The early stages in the formation of the end sac out of the fifth thoracic somite have already been described.

Before the Trilobite stage a longitudinal section shows the presence of a few enlarged finely granular cells in the walls of the end sac. These granules increase in size and numbers till the cells present the appearance shown in Pl. XXIV, Fig. 42, *c.s.* They now resemble those cells from which the nephridial tubules develop.

In the Trilobite stage (Pl. XXVIII, Fig. 80) the coarse granular protoplasm has nearly disappeared, and the sac is lined with a delicate layer of protoplasm, with here and there a nucleus. Numerous finger-like evaginations of the wall of the sac have developed, the walls of which have the same structure as those of the sac itself.

It was not possible to determine the exact manner in which these evaginations were formed. A careful study indicates that the large tubes opening directly into the sac were formed as











evaginations of the walls of the sac, and that the outgrowths were subsequently increased in length by the addition of nephridial cells to their distal ends. These cells in turn become hollowed out and united with the cells forming the nephric lobes. In this way the system of tubules in the nephric lobes becomes continuous with those leading into the end sac.

There seem to be two sets of tubules opening into the sac; one set arises from its anterior wall and leads into the longitudinal tubules of the stolon, and hence to the three anterior nephric lobes; the others lead into the tubules of the fourth lobe. On its median side the sac opens through a small neck into the nephric duct.

Finally, in the adult, the end sac becomes so irregular through the formation of the numerous large tubes opening into it that its original boundaries cannot be distinguished.

C. *The nephric duct of the adult* lies along the edge of the plastron dorsal to the nephric lobes, and extends backwards from the base of the second leg to the anterior side of the sixth. The thin-walled transparent tube is easily torn, and, unless injected, it is very difficult to trace out its various convolutions. On careful dissection the course of the duct is seen to be as follows: At the distal end of the duct, just before it opens to the exterior, is the ectodermic portion (Pl. XXVIII, Fig. 83, *ect.* and cut 3 B.). It is sharply marked off from the rest of the duct by its thick walls lined with chitin. From this point the duct turns at right angles and extends in a dorsal direction, till it reaches the plastron, along the lateral edge of which it extends as far as the first nephric lobe (Pl. XXVIII, Fig. 83). It then bends directly backwards, diminishing rapidly in size up to the angle of the second loop, which in Pl. XXVIII, Fig. 83, is seen on the median side of the fourth nephric lobe. From here on the calibre of the tube remains about the same. It now turns forwards, parallel to the dorsal limb of the loop, as far as the posterior margin of the third lobe, and then backwards to form a large mass of coils, lying a little behind and dorsal to the fourth lobe. From this coil the proximal end of the duct issues and passes forwards and ventrally to the end sac, buried in the interior of

the fourth lobe. From the end sac many tubes lead forward into the stolon. The latter consists of a coarse network of anastomosing tubes, from which branches are given off that extend along the median dorsal face of each lobe, diminishing in size as they go. The entire substance of the lobes may be colored a deep red by injecting red gelatine into the main duct.

Along the walls of the duct are here and there short, blunt evaginations or pockets ending blindly. In some cases the pockets of one tube may unite with those of another, thus forming communications between the separate coils (Pl. XXVII, Figs. 81 and 82, *po.* and *c.n.t.*). They are most numerous in the extensive coil lateral to the fourth nephric lobe.

#### IX. CELLS OF DOUBTFUL SIGNIFICANCE.

During the Trilobite stage certain cells appear on the nephridial lobes, which may be readily recognized by peculiarities of shape and coloring (Pl. XXV, Fig. 45; Pl. XXVIII, Figs. 74 and 80, *s.r.c.*). They are dark purple when stained in Lyon's blue and borax carmine. These cells were found only in the vicinity of the hollow nephridial tubules, with which they were often so closely connected that it was impossible to ascertain with certainty whether they were inside or outside the tubules. Occasionally they were on the outer margin of the tubules, and it would then appear as if they were about to separate from them (Pl. XXVIII, Fig. 74, *c.*). In Pl. XXVIII, Fig. 80, at the dorsal side of the end sac, about a dozen were collected, which suggested a point of proliferation either by cell division among themselves or from the nephridial tubules or sac. At the left (*v.c.*) one of the cells is much larger than the others and shows vacuolations in the protoplasm. A few of these cells were found in the second larval stage, as shown in Pl. XXV, Fig. 46, *r.c.*, and also in the region of the heart (Pl. XXVIII, Fig. 76, *r.c.*), after which they entirely disappeared. A large number of granular cells appear at this time, and it seems probable that they are different conditions of the same cells, although no convincing proof of it could be found.

The last-named cells may be found in the second larval stage distributed throughout the nephridial lobes (Pl. XXV, Fig. 47, *g.c.*), and they occur in large masses along their lateral margins. These cells are round or oval, and are filled with great numbers of coarse granules, which usually completely conceal the nucleus. They are well shown in Pl. XXV, Fig. 46, *g.c.*, and Pl. XXVIII, Fig. 75, *g.c.*

In larvae three-quarters of an inch long the cells on the lateral margins of the lobes are enormous (Pl. XXV, Fig. 48, and Pl. XXVIII, Fig. 77). Similar cells were found throughout the body, from the proventriculus to the first gill. In the anterior sections they are most numerous on the dorsal and lateral sides of the proventriculus. They also extend laterally on the ventral side of the body close to the ectoderm. Posterior to this they are less abundant around the alimentary canal, but are thickly massed around the base of the legs. In the sixth leg and in the operculum they are more numerous than in any other place. Similar cells are found in the region of the heart. The origin and fate of these cells were not determined with certainty. They agree in some respects with the granular cells seen in the early stages of the nephric lobes, and which, as we have seen, subsequently cleared up and formed the nephric tubules.

During the Trilobite and second larval stage, cells are found in the pericardial region that closely resemble nephridial cells. They are most abundant on the dorsal side of the pericardium in the sixth thoracic segment and over the proventriculus. Many cells are hollow and united end to end, forming loose-branching tubules like those in the nephridial lobes of the Trilobite stage (Pl. XXVIII, Fig. 76, *h.c.t.*). Among these cells are a few of the large granular ones (*g.c.*<sup>3</sup>), and some of the small dark red cells (*r.c.*) like those seen in the nephridial lobes. All these cells probably arose from the nephridial "Anlagen" at the base of the legs, and were carried to their present position by the growth of the somites over the dorsal surface of the egg.

The same kind of cells are also found in the chelicerae and in the sixth leg. Those in the chelicerar segment (Pl. XXIV, Fig. 40) disappear early. Those in the sixth leg appear before

the Trilobite stage (Pl. XXV, Fig. 43, *n.c.*<sup>6</sup>). These cells first become granular, then vacuolated, and then united end to end (Pl. XXVIII, Fig. 79). In Limuli about three-quarters of an inch long the cells were still present, but they were not united with the permanent nephric lobes, and appeared to be degenerating.

#### X. SUMMARY.

1. *Branchial Cartilages*.—A thick ring of somatic mesoderm forms at the base of each abdominal appendage. The gill cartilage arises as a plate of somatic mesoderm attached by its dorsal end to the ventral wall of the somite, and continuous on either side with the ring of mesoderm. The ventral end of the cartilage finally extends through and beyond the mesodermic ring and becomes attached to the anterior wall of the corresponding appendage.

2. The ventral ends of the abdominal somites persist as venus sinuses.

3. The *genital ducts* arise as diverticula of the median ventral side of the opercular somite. They remain in a rudimentary condition until after the second larval stage.

4. *Nephric Duct*.—A nephric plate is formed from a single layer of columnar cells of the somatic mesoderm on the median side of the fifth somite. The plate is gradually evaginated to form a funnel, opening by a wide mouth into a thin-walled end sac that represents the fifth somite; the opposite end unites with a shallow ectodermic invagination at the base of the fifth leg. The tube becomes much convoluted, and is converted directly into the adult nephric duct. Finger-like outgrowths of the end sac finally unite with the hollow cell chains of the adjacent nephric lobes.

5. *The Nephric Lobes*.—A mass of nephric cells arises independently of the duct from the median dorsal portion of the somatic layer of each of the six thoracic somites. The cells become enlarged and filled with coarse granules. The granules become smaller or disappear, and a vacuole appears in each cell. The latter elongate and unite end to end to form irregular masses of branching intracellular tubules. The cell masses



in the second, third, fourth, and fifth legs form the four lobes of the adult organ. Offshoots extend forward and backward from the median end of each lobe, that unite with each other and with the end sac to form the longitudinal ducts of the stolon. The nephric cells of the first and sixth somite disappear. In the stolon, and in the larger tubules on the dorsal side of each lobe, the nephric cells become flattened, and by repeated divisions are finally converted into a pavement epithelium, thus changing the intracellular lumina into intercellular ones. New tubules are formed throughout life by the transformation of indifferent cells on the ventral surface of each lobe.

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## INDEX LETTERS TO PLATE XXII.

<i>a.p.</i> = anal plate.	<i>p.f.</i> = primitive furrow.
<i>B.c.</i> = blood corpuscles.	<i>p.s.</i> = primitive streak.
<i>ch.</i> = chelaria.	<i>SO</i> <sup>0</sup> = unexplained cavity appearing only in this series.
<i>c.r.</i> = opercular cartilage.	<i>SO</i> <sup>1-5</sup> = somite of the chelaria, operculum, and first to third gills, respectively.
<i>ect.</i> = ectoderm.	
<i>g.</i> <sup>1</sup> = first gill.	
<i>g.d.</i> = genital duct.	
<i>op.</i> = operculum.	

## EXPLANATION OF PLATE XXII.

All the sections were outlined with a camera and drawn to the same scale.

FIGS. 1-8 were drawn from a series of longitudinal sections through the region of the chelaria (*ch.*), operculum (*op.*), and the first gill (*g.*<sup>1</sup>) of an embryo in which the abdominal appendages were beginning to show in surface views. Borax carmine, 15  $\mu$ .

FIG. 1. Section No. 1, through the median line of the embryo. A few scattered mesoderm cells lie between the ectodermic layer and the yolk. There were also a very few large nuclei in the yolk, and others from which chromatin granules were escaping, as if they were beginning to degenerate.  $\times 200$ .

FIG. 2. Section No. 2. The median ends of the somites are shown by four bunches of cells. *SO*<sup>1</sup> is the somite of the chelaria; *SO*<sup>2</sup>, that of the operculum; *SO*<sup>3</sup>, one which disappeared shortly after this stage; *SO*<sup>3</sup>, somite of the first gill about to separate from the primitive streak (*p.s.*).  $\times 200$ .

FIG. 3. Section No. 3. The somatic cavities are distinct; *SO*<sup>0</sup> merges with the ectoderm on the ventral side of the somite.  $\times 200$ .

FIG. 4. Section No. 6. *SO*<sup>1</sup>, *SO*<sup>2</sup>, and *SO*<sup>3</sup> are larger than in the previous sections. The cavity of *SO*<sup>0</sup> has disappeared, and in its place are a few mesoderm cells which disappear in the next section.  $\times 200$ .

FIG. 5. Section No. 11. The operculum and the first gill are much larger than formerly, and are now filled with mesoderm. Their somatic cavities remain distinct.  $\times 200$ .

FIG. 6. Section No. 13. *SO*<sup>2</sup> shows a diverticulum, the beginning of the genital duct (*g.d.*).  $\times 200$ .

FIG. 7. Section No. 18. *SO*<sup>2</sup> remains large. *SO*<sup>3</sup> has almost closed.  $\times 200$ .

FIG. 8. Section No. 23. *SO*<sup>2</sup> is the only abdominal somite remaining; its lumen disappears a few sections farther toward the lateral side.  $\times 200$ .

FIGS. 9-20 were drawn from a series of longitudinal sections through the region of the chelaria, operculum, and the first gill of an embryo somewhat older than the one in the preceding series. In this embryo there were four perfect abdominal somites; the fifth was just breaking free from the primitive streak. The series

begins with Fig. 9 near the median line and extends laterally almost to the outer edge of the appendages. Borax carmine and Lyon's blue, 10  $\mu$ .

FIG. 9. Section No. 1, near the median line, showing five abdominal somites,  $SO^{1-5}$ .  $SO^5$  is attached to the primitive streak (*p.s.*); the point of separation is indicated by a slight furrow on the dorsal side. The enlargement at the anterior end of the primitive streak will form the sixth somite.  $\times 200$ .

FIG. 10. Section No. 3.  $SO^5$  extends in a lateral direction beyond the point of attachment to the primitive streak.  $SO^{1-4}$  are distinct and free from the mesoderm at the base of the appendages.  $\times 200$ .

FIG. 11. Section No. 6. The median end of the genital duct (*g.d.*) may be seen in the operculum.  $SO^2$  is small and lies close to the surface of the yolk.  $\times 200$ .

FIG. 12. Section No. 7. The genital duct is larger than in the previous section, and is nearer  $SO^2$ .  $\times 200$ .

FIG. 13. Section No. 12. The genital duct and  $SO^2$  are in contact. In the first gill the mesoderm cells are arranged in a row, extending down into the mass of mesoderm from the ventral side of the somite to form the cartilage (*c.r.*) of the first gill.  $\times 200$ .

FIG. 14. Section No. 13. The cavity of  $SO^2$  and the genital duct are separated by a thin membrane only.  $\times 200$ .

FIG. 15. Section No. 14.  $SO^2$  and *g.d.* have united. A deep furrow on the anterior side indicates the point of union.  $\times 200$ .

FIG. 16. Section No. 17. A line of cells on the ventral margin of the opercular somite shows the median limit of the opercular cartilage.  $\times 200$ .

FIG. 17. Section No. 20. The opercular cartilage is free from the mesoderm, and consists of a single row of cells on the ventral side of  $SO^2$ .  $\times 200$ .

FIG. 18. Section No. 22. The end of the cartilage rod is broader than before, and a thin membrane connects it with the adjacent mesoderm.  $\times 200$ .

FIG. 19. Section No. 26. The opercular cartilage has disappeared. The ventral margin of the somite connects with the posterior bunch of mesoderm cells. The somites show a tendency to bend in a posterior direction.  $\times 200$ .

FIG. 20. Section No. 32.  $\times 200$ .

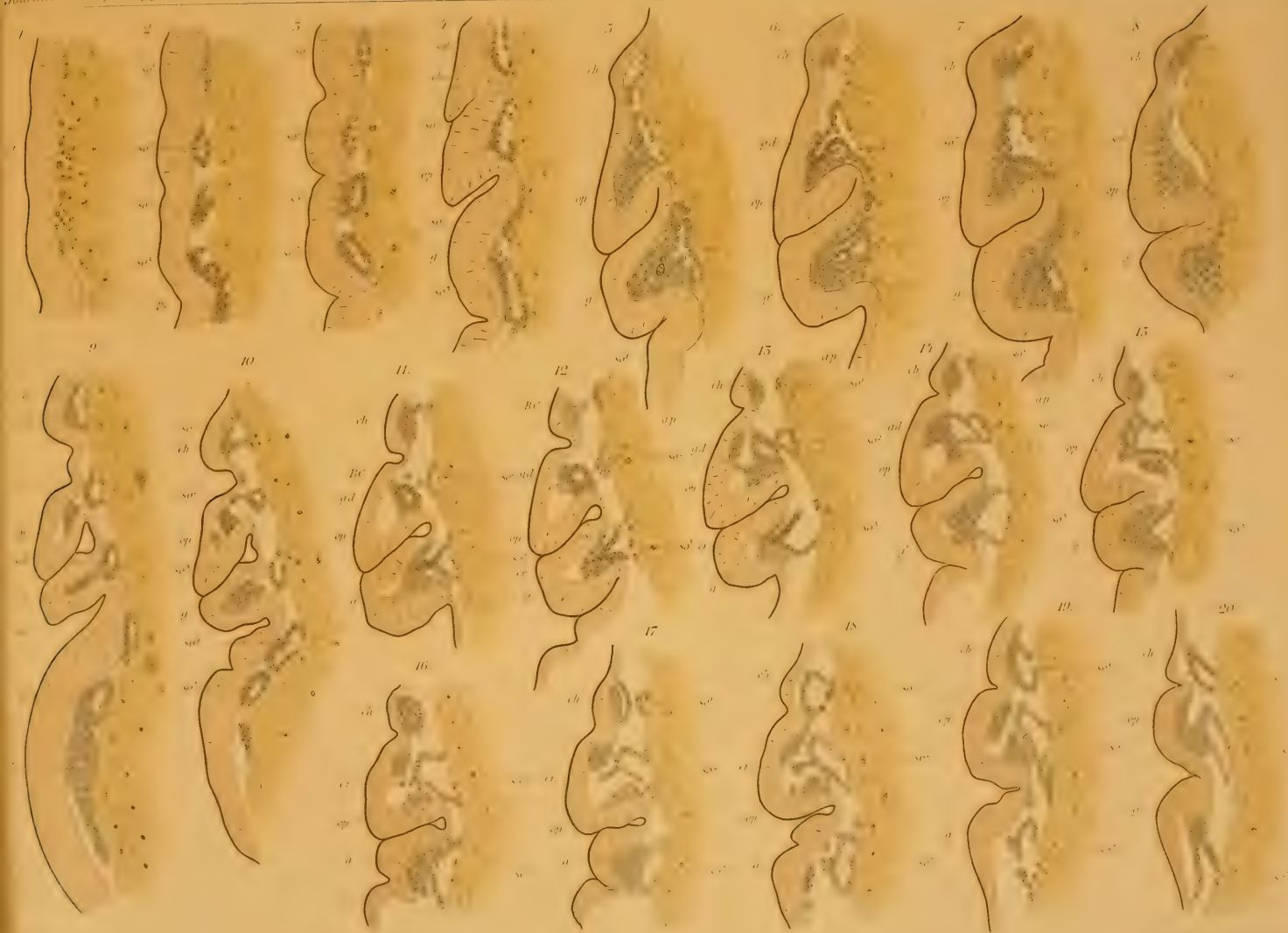














INDEX LETTERS TO PLATE XXIII.

<i>B.c.</i> = blood corpuscles.	<i>n.</i> = nerve.
<i>Ch.</i> = chelaria.	<i>n.p.</i> = proliferation of nerve cells and fibers.
<i>c.r.</i> = cartilage rod.	<i>o.p.</i> = operculum.
<i>cu.</i> = cuticular membrane.	<i>SO</i> <sup>1</sup> = somite of the chelaria.
<i>ec.p.</i> = ectodermic proliferation.	<i>SO</i> <sup>2</sup> = somite of the operculum.
<i>ect.</i> = ectoderm.	<i>SO</i> <sup>3</sup> = somite of the first gill.
<i>end.</i> = endothelium.	<i>v.s.</i> = venous sinus.
<i>g.'</i> = first gill.	<i>x.</i> = fusion of cartilage, ectoderm, and mesoderm.
<i>g.d.</i> = genital duct.	<i>y.k.</i> = yolk.
<i>m.</i> = muscle cells.	
<i>ms.</i> = mesoderm.	

EXPLANATION OF PLATE XXIII.

FIGS. 21-24 represent longitudinal sections through the region of the chelaria, operculum, and first gill of an embryo a little more advanced than the one from which Figs. 9-20 in Plate XXII were drawn. The sections were 15  $\mu$  thick and stained in borax carmine and Lyon's blue.

FIG. 21. Section No. 1, near the median line. The somites of the operculum and the first gill have extended so far in a lateral direction that the somite of the chelaria could not come in the same longitudinal sections with them, but would be found in the sections nearer the median line. The somites of the operculum (*o.p.*) and the first gill (*g.'*) are large and distinct. The genital duct (*g.d.*) shows at the base of the operculum. The lumen seen here, ends in a solid mass of mesoderm in the preceding section.  $\times 200$ .

FIG. 22. Section No. 5. The genital duct is larger than in the preceding section, and has approached *SO*<sup>2</sup>.  $\times 200$ .

FIG. 23. Section No. 10. The genital duct and *SO*<sup>2</sup> have united. A few cartilage cells (*c.r.*) are seen on the dorsal wall of the somite.  $\times 200$ .

FIG. 24. Section No. 10. Both *SO*<sup>2</sup> and *SO*<sup>3</sup> show a tendency to bend in a posterior direction. The opercular cartilage (*c.r.*) is still present.  $\times 200$ .

FIGS. 25-29. Longitudinal sections through the region of the operculum and the first gill from an embryo with two gill leaves on the first gill. The sections were 15  $\mu$  thick and stained with borax carmine and Lyon's blue.

FIG. 25. Section No. 1, near the median line. The opercular cartilage and genital duct extend toward the median line some distance beyond the somite. The opercular cartilage is attached to the genital duct between bunches of mesoderm at the base of the operculum. The first gill cartilage is now visible. In the spaces between the ectoderm and mesoderm of the appendages are a few blood corpuscles (*B.c.*). Some of the mesoderm cells on the yolk show muscular

striations (*m.*). At the posterior side of the first gill are nerve fibers beneath the place where the first gill leaf is forming (*n.*).  $\times 200$ .

FIG. 26. Section No. 5. The genital duct and opercular cartilage are larger than in the preceding drawing.  $SO^2$  shows at the posterior side of the operculum.  $SO^3$  is greatly enlarged.  $\times 200$ .

FIG. 27. Section No. 8. The genital duct (*g.d.*) and  $SO^2$  are separated by a thin membrane.  $\times 200$ .

FIG. 28. Section No. 10. The genital duct and  $SO^2$  have united. The opercular cartilage remains on the dorsal side of the somite.  $SO^2$  and  $SO^3$  show a tendency to extend in a posterior direction.  $\times 200$ .

Fig. 29. Section No. 19. The somites extend a long distance laterally and posteriorly as closed cavities.  $\times 200$ .

FIGS. 30-33. Longitudinal sections through the operculum and first branchial appendage of an embryo in which the third gill leaf had commenced to form. The embryo appears older than that of the preceding series as the appendages have lengthened considerably.

FIG. 30. Section No. 1, near the median line. Shows the median end of the genital duct at the base of the operculum. As the somite has grown laterally and the genital duct toward the median line, they no longer appear in the same longitudinal sections. Nerve fibers (*n.*) are found at the posterior side of the base of the operculum and the first gill.  $\times 200$ .

FIG. 31. Section No. 3. Shows the genital duct with a small lumen and the median edge of the opercular cartilage.  $\times 200$ .

FIG. 32. Section No. 10. Shows the lateral end of the genital duct connected with the base of the opercular cartilage. The genital duct is relatively much smaller than in the preceding series and separate from the somite ( $SO^2$ ). The first gill has a well-formed cartilage.  $\times 200$ .

FIG. 33. Section much farther from the median line. It shows the long, slender cartilage plates; that of the first gill is attached to the ectoderm on the anterior side of the appendage. A similar condition would be found in other sections in the operculum. The cartilage cells are placed in rows and show a characteristic appearance, and take a lighter stain than the mesoderm. The cartilages are surrounded by a thick membrane. Each somite has been transformed into a large venous sinus, and extends from the base of the cartilage through the yolk to the dorsal side of the embryo. A number of nerve fibers and cells (*n.*) are shown in the gill leaf in *g.*  $\times 200$ .

FIG. 34. Longitudinal section through the operculum and the first gill of a specimen with five gill leaves on the first gill, showing the opercular cartilage attached to the anterior wall of the operculum and to the venous sinus at the base of the appendage. The cartilage is surrounded by a membrane, the perichondrium (*pc.*). At the apex of the leg slender processes reach from one ectodermic wall to the other. The outer wall is covered by a thin cuticular membrane.  $\times 200$ .









INDEX LETTERS TO PLATE XXIV.

<i>ap.<sup>2-6</sup></i> = the second to the sixth thoracic appendages.	<i>m.r.</i> = marginal ring.
<i>b.c.</i> = blood corpuscle.	<i>n.c.</i> = nephridial cells.
<i>br.</i> = brain.	<i>n.c.<sup>1-5</sup></i> = nephridial cells of the first to the fifth appendages, respectively.
<i>b.s.</i> = blood space.	<i>ped.n.</i> = pedal nerve.
<i>b.v.</i> = blood vessel.	<i>s.c.</i> = sensory cells.
<i>che.</i> = chelicera.	<i>SO</i> = somite.
<i>ect.</i> = ectoderm.	<i>sop.</i> = somatopleure.
<i>e.s.</i> = end sac.	<i>sp.c.</i> = spinal cord.
<i>g.n.c.</i> = granular nephridial cells.	<i>spl.</i> = splanchnopleure.
<i>mes.</i> = mesoderm.	<i>yk.</i> = yolk.

EXPLANATION OF PLATE XXIV.

FIGS. 35-39 were drawn from cross-sections through corresponding regions of the third and fourth thoracic appendages of embryos of varying ages. The sections are all arranged so that the median line is at the upper margin of the plate. The sections of the younger embryos are  $3\mu$  to  $5\mu$  thick, the older ones  $10\mu$ .

FIG. 35. Cross-section through the middle of the fourth appendage of an embryo in which none of the abdominal appendages had been formed. On the dorsal margin of the mass of mesoderm lying near the median side of the base of the appendage may be seen four or five larger cells, the "Anlage" of the nephridial lobe (*n.c.*).  $\times 200$ .

FIG. 36. Cross-section through the middle of the third appendage of an embryo slightly older than that in Fig. 35. The nephridial cells are more numerous, larger, and have faint granulations. A thin non-cellular membrane covers the yolk at the base of the appendage. A space is formed between the mesoderm and the apex of the appendage, which later develops into the blood space of the legs.  $\times 200$ .

FIG. 37. Cross-section through the middle of the fourth appendage of an embryo older than that in Fig. 36. The somites are imperfectly formed in the thoracic appendages. The somatic layer is several cells thick; the splanchnic layer is represented by a thin membrane with a few nuclei. The nephridial cells are larger than in the preceding figures. They possess slender pseudopodia, have become finely granular, and take a deep stain in Lyon's blue.  $\times 200$ .

FIG. 38. Drawn from the same series and the fourth section back of that in Fig. 37. It shows the nephridial cells smaller than at the middle of the base of the appendage and without pseudopodia. The somite extends out laterally as a closed cavity.  $\times 200$ .

FIG. 39. Cross-section through the middle of the fourth appendage. Large granular cells with long processes are shown on the dorsal margin of the meso-

derm. The largest of these cells lie beyond the lateral base of the appendage.  $\times 200$ .

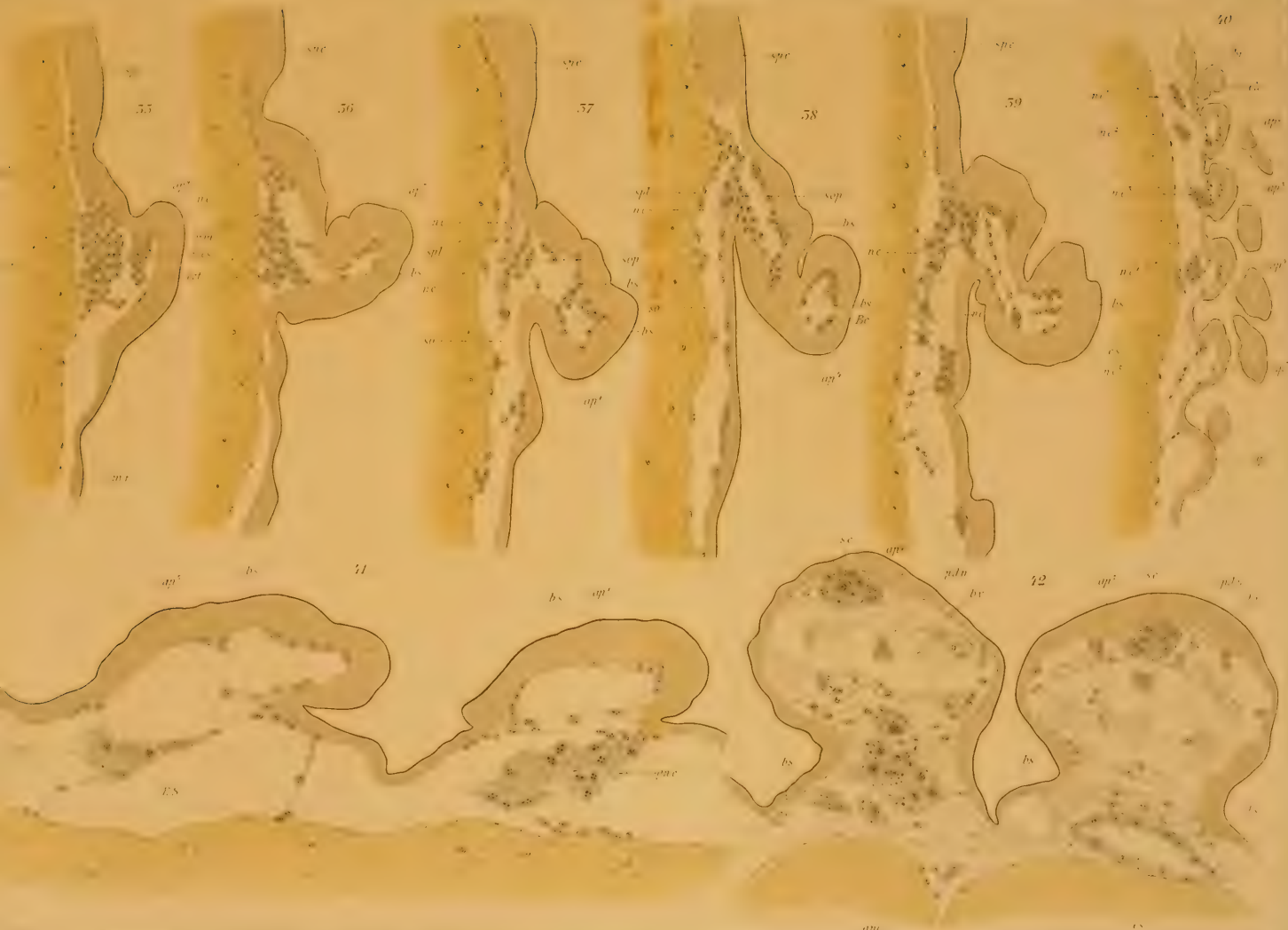
FIG. 40. A longitudinal section from an embryo of about the same age as that in Pl. XXIII, Figs. 30-32. The somites of the thorax have disappeared, except the one in the fifth appendage, which remains as the end sac to the nephridial duct. Bunches of nephridial cells are found in the chelicerae and in the second, third, and fourth appendages. Nephridial cells appear later in the sixth appendage.  $\times 100$ .

FIG. 41. Longitudinal section through the fourth and fifth appendages. The nephridial cells are filled with large granules, among which the larger nuclei are visible. Often the cell boundaries were very indistinct or else entirely invisible, giving the appearance of several nuclei in the same cell. In the fifth appendage the end sac shows as a closed cavity, with a few of the larger nephridial cells on its ventral wall.  $\times 400$ .

FIG. 42. Longitudinal section through the fourth and fifth legs. It shows a bunch of large, granular, nephridial cells at the base of the fourth leg. In the fifth leg the end sac is lined with granular cells which are similar to those in the fourth leg, except that they are smaller. There is a blood space between the appendages and at the apex of the appendages.  $\times 300$ .











INDEX LETTERS TO PLATE XXV.

<i>ap.<sup>2-6</sup></i> = the second to the sixth appendages, respectively.	<i>l.n.c.</i> = longitudinal section through nephridial cells.
<i>ant.n.d.</i> = anterior arm of the nephric duct.	<i>n.c.<sup>1-6</sup></i> = nephridial cells in the first to the sixth appendages, respectively.
<i>b.r.</i> = brain.	<i>n.c.</i> = nephridial cell.
<i>b.s.</i> = blood space.	<i>n.d.</i> = nephric duct.
<i>b.v.</i> = blood vessel.	<i>nl<sup>3</sup>, nl.<sup>4</sup></i> = third and fourth nephridial lobes.
<i>c.n.c.</i> = cross-section through nephridial cell.	<i>nt.</i> = nephric tubules.
<i>e.s.</i> = end sac.	<i>oe.</i> = oesophagus.
<i>ex.n.d.</i> = nephric duct near the external opening.	<i>pl.</i> = plastron.
<i>g.c.</i> = granular cells.	<i>post.n.d.</i> = posterior arm of the nephric duct.
<i>g.n.c.</i> = granular nephridial cells.	<i>r.c.</i> = red cells.
<i>g.t.</i> = tubules lined with granule cells.	<i>a.t.</i> = areolar tissue.

EXPLANATION OF PLATE XXV.

FIG. 43. Longitudinal sections through the second to the sixth legs of an embryo a little younger than the Trilobite stage. The chelicera have remained near the median line, so that they are not included. Bunches of nephridial cells are seen at the base of the second, third, fourth, and sixth legs, and the nephric duct in the fifth leg.  $\times 100$ .

FIG. 44. A transverse section through the region of the second, third, and fourth legs of the Trilobite stage. Nephridial cells are seen at the base of the third and fourth legs. The duct has grown anteriorly nearly as far as the second leg, and both the distal and proximal limbs are shown in the figure.  $\times 100$ .

FIG. 45. Enlarged drawing through the dorsal region of the fourth and fifth legs from the same series as Fig. 44, showing the nephric duct and the nephridial cells in various stages of development.  $\times 400$ .

FIG. 46. Horizontal section through the fourth and fifth legs of a specimen in the second larval stage. As the section was cut near the external opening of the duct, only one portion of it is seen. The nephridial lobes form a definitely marked area, and the portions in the second, third, fourth, and fifth legs have united with one another. The nephridial lobes are composed of a lacuna tissue, in which are large granular cells of varying sizes.  $\times 200$ .

FIG. 47. Cross-section through the fourth leg of a younger specimen of the second larval stage than the preceding one. The dorsal side of the section is on the right. The nephric duct is much coiled, and several sections through it are shown. Cylindrical cells with fine granules around the periphery are uniting end

to end, forming long, narrow tubules. These tubules are found on the median and dorsal side of the nephridial lobes. A number of small cells with large granules (*g.c.*) show on the lateral side of the lobes.  $\times 300$ .

FIG. 48. Cross-section through the fifth leg of a crab about one inch long. The dorsal side of the drawing is on the right and the median at the lower margin of the page. It shows sections through the nephridial duct and the end sac with its long, branching diverticula. The structure of the lobe differs in different places. Near the end sac it is composed of long, branching tubules, lined with cells containing coarse granules. Outside this layer the tubule cells are smaller; on the lateral side of the lobe the tissue is aerolated. On the lateral margin are large cells filled with very coarse granules.  $\times 100$ .











INDEX LETTERS TO PLATE XXVI.

<i>a.</i> = anterior.	<i>m.</i> = median.
<i>art.</i> = artery.	<i>m.art.</i> = main artery.
<i>c.</i> = cells filled with fine granules.	<i>mes.</i> = mesoderm.
<i>cap.</i> = capillaries.	<i>m.r.</i> = marginal ring.
<i>ctis.</i> = connective tissue.	<i>m.y.</i> = membrane on the yolk.
<i>c.t.</i> = collecting tubules.	<i>n.</i> = nerve.
<i>e.n.d.</i> = evagination of nephric duct.	<i>n.c.</i> = nerve cord.
<i>e.s.</i> = end sac.	<i>n.d.</i> = nephridial duct.
<i>ex.op.</i> = external opening of the nephric duct.	<i>n.f.</i> = nerve fibers.
<i>f.g.c.</i> = fine granular cells.	<i>p.</i> = posterior.
<i>g.c.</i> = granular cells.	<i>p.n.d.</i> = nephric plate.
<i>g.t.</i> = granular tubules.	<i>s.l.</i> = striated layer.
<i>h.c.</i> = hollow cells.	<i>so.</i> = somite.
<i>int.n.</i> = integumentary nerve.	<i>t.</i> = tubules.
<i>l.</i> = lateral.	<i>t.p.</i> = tubular portion.
	<i>y.k.</i> = yolk.

EXPLANATION OF PLATE XXVI.

FIG. 49. Longitudinal section through the middle of the second lobe of an adult nephridium. The section was  $5\mu$  thick and was stained in borax carmine and Lyon's blue. The median side of the lobe is at the right. The lobe is composed of four distinct regions or layers, each of which has a characteristic structure.  $\times 16$ . (1) The outer portion is formed of very large granular cells (*g.c.*) (see Fig. 50) most abundant at the lateral end of the lobe on both dorsal and ventral sides. Two smaller groups are shown on the median side. Small nerve fibers penetrate this tissue. (2) *h.c.* A dark layer which surrounds the lobe, except for a short distance on its median ventral side. It is composed of small cells with fine granules around the periphery. They are probably hollow cells (*h.c.*) which are uniting end to end (see Fig. 51). (3) *g.t.* A faintly stained layer inside of *h.c.* and surrounding the entire lobe. It is composed of small tubules which are lined with large granular cells (see Fig. 52). (4) The larger part of the lobe is contained in this tubular portion (*t.p.*) (see Fig. 53). (5) *c.t.* The collecting tubular portion is similar to (4), except that the tubules are larger. They connect the small tubules of each lobe with the collecting tubes of the stolon. It is shown on the dorsal side of the figure and near the median end of the lobe (see Fig. 54).

FIG. 50. An enlarged drawing through the large cells which surround the lateral end of Fig. 49 (*g.c.*). They are filled with small granules, which did not stain in either borax carmine and Lyon's blue, or Delafield's haematoxylin and eosin. Several nuclei are often found in the same cell. Nerve fibers penetrate

throughout the tissue. A bunch of small, round cells, with fine granules around their periphery (*fg.c.*), is seen, apparently within the large cell on the left.  $\times 270$ .

FIG. 51. Some of the hollow cells which nearly surround the nephridial lobe (*h.c.*, Fig. 49). It shows cells with a finely granular periphery, apparently uniting end to end.  $\times 270$ .

FIG. 52. Enlarged section through the tubules lined with large granular cells.  $\times 270$ .

FIG. 53. Enlarged section of the tubular part (*t.p.*) of the lobe. The tubules are surrounded by a loose connective tissue, in which not infrequently were large, round cells with granular protoplasm (*c.*). The tubules form an anastomosing network throughout the central portion of the lobe. They have a cellular lining which is separated by a dark membrane from the connective tissue (*ctis.*).  $\times 270$ .

FIG. 54. Enlarged portion of the longitudinal collecting tubules (*ct.*). The tubules are large and branching. They have a heavily striated lining, which is separated by a dark membrane from the connective tissue. Nerves and capillaries are shown in the connective tissue, and also a few large granular cells, similar to those in Fig. 53.  $\times 270$ .

FIG. 55. Cross-section through the anterior part of the fifth appendage of a specimen the same age as that in Pl. XXIV, Fig. 36 ( $5\mu$ , haematoxylin). The section shows the nephric plate of mesoderm cells at the base of the appendage. It is continuous on both the lateral and median sides with a cellular membrane which lies upon the yolk.  $\times 200$ .

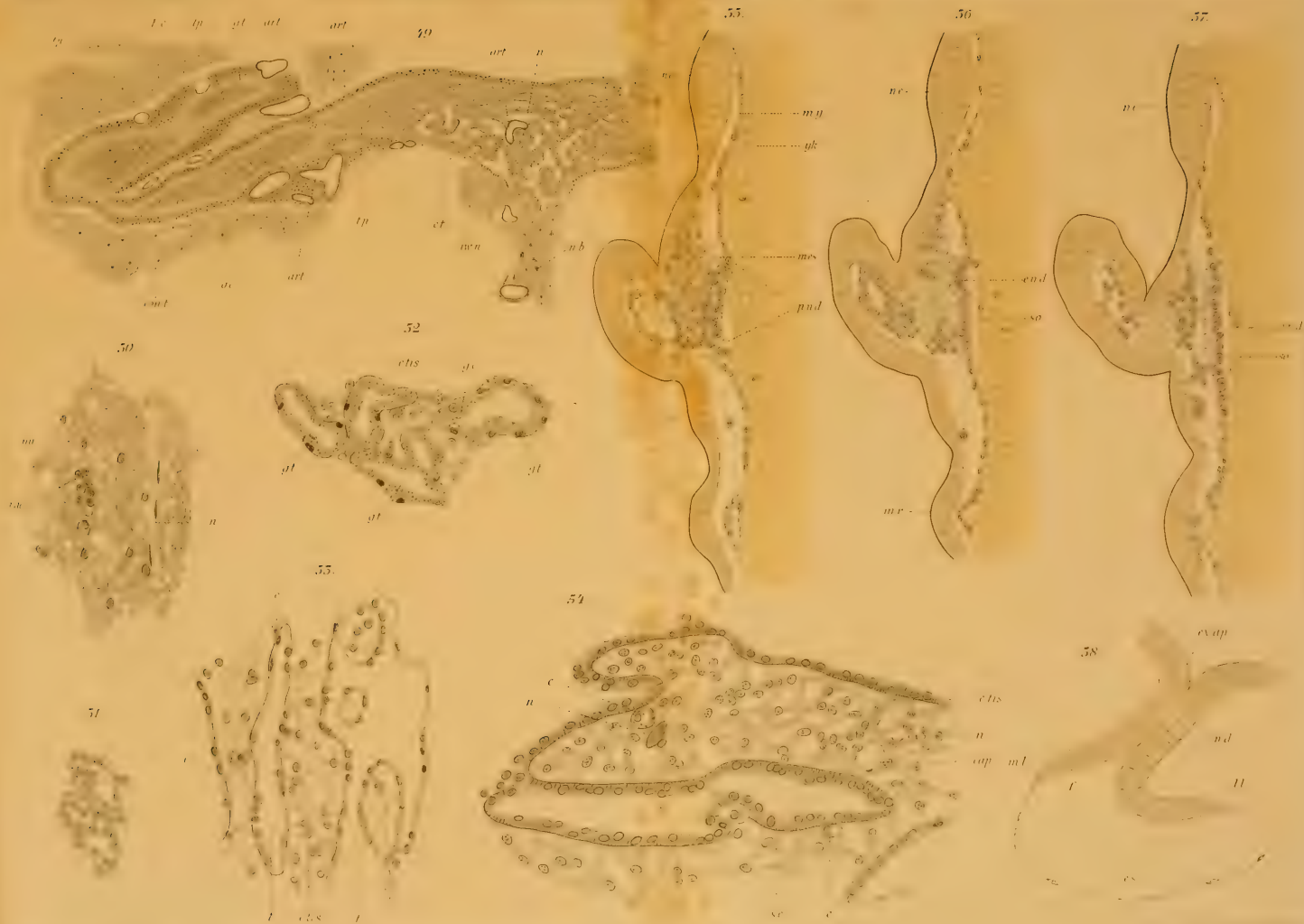
FIG. 56. The fourth section posterior to Fig. 55, showing an evagination of the nephric plate to form the nephric duct.  $\times 200$ .

FIG. 57. The third section posterior to Fig. 56, showing the posterior margin of the nephric plate.  $\times 200$ .

FIG. 58. Reconstructed outline of the nephridial duct and end sac, made from a specimen of the same age as Pl. III, Fig. 40.  $\times 400$ .











INDEX LETTERS TO PLATE XXVII.

<i>ap.</i> <sup>5</sup> = fifth appendage.	<i>mes.</i> = mesoderm.
<i>ect.p.</i> = proliferation of ectoderm.	<i>m.r.</i> = marginal ring.
<i>e.n.d.</i> = ectodermic portion of nephridial duct.	<i>n.c.</i> = nerve cord.
<i>e.s.</i> = end sac.	<i>n.d.</i> = nephric duct.
<i>g.n.c.</i> = granular nephridial cells.	<i>p.n.d.</i> = nephric plate.
<i>l.n.d.</i> = lip of the nephric duct.	<i>s.m.</i> = sphincter muscle.
	<i>so.</i> = somite.

EXPLANATION OF PLATE XXVII.

FIGS. 59-64 were drawn from a series of cross-sections through the fifth appendage of an embryo of the same age as that in Pl. XXIV, Figs. 37 and 38. The sections were cut 8  $\mu$  thick and stained with Delafield's haematoxylin.

FIG. 59. Section No. 1, showing a mass of mesoderm cells at the base of the appendage, in the middle of which are a few larger and lighter-colored cells which mark the anterior margin of the nephric duct.  $\times 200$ .

FIG. 60. Section No. 2, showing the nephric plate folded on itself, making a double layer of large, clear cells, which extend toward the ectoderm. The somite is a closed cavity dorsal to the nephridial duct.  $\times 200$ .

FIG. 61. Section No. 4, showing the nephric duct extending out to the ectoderm on the median margin of the appendage.  $\times 200$ .

FIG. 62. Section No. 6. The nephric duct and somite are much reduced in size, but retain the same relative position as before.  $\times 200$ .

FIG. 63. Section No. 7, showing the somite reduced to a long, narrow space on the surface of the yolk. In place of the nephric duct is a row of large mesoderm cells with a slight outward projection.  $\times 200$ .

FIG. 64. Section No. 10. The nephric duct is represented by a row of large cells, continuous on the median and lateral sides with the yolk membrane. The mesoderm at the base of the appendage and the somite have entirely disappeared.  $\times 200$ .

FIGS. 65-70 are drawn from a series of cross-sections through the fifth appendage of an embryo somewhat older than the one in the preceding series. The proximal end of the duct has grown away from the median line, changing its general direction somewhat.

FIG. 65. Section No. 1 shows the anterior margin of the nephric plate.  $\times 200$ .

FIG. 66. Section No. 3 shows the mouth of the duct opening with a broad lateral lip on the ventral side of the somite.  $\times 200$ .

FIG. 67. Section No. 5 shows cross-section through the middle of the duct.  $\times 200$ .

FIG. 68. In Section No. 9 the lumen of the duct has disappeared.  $\times 200$ .

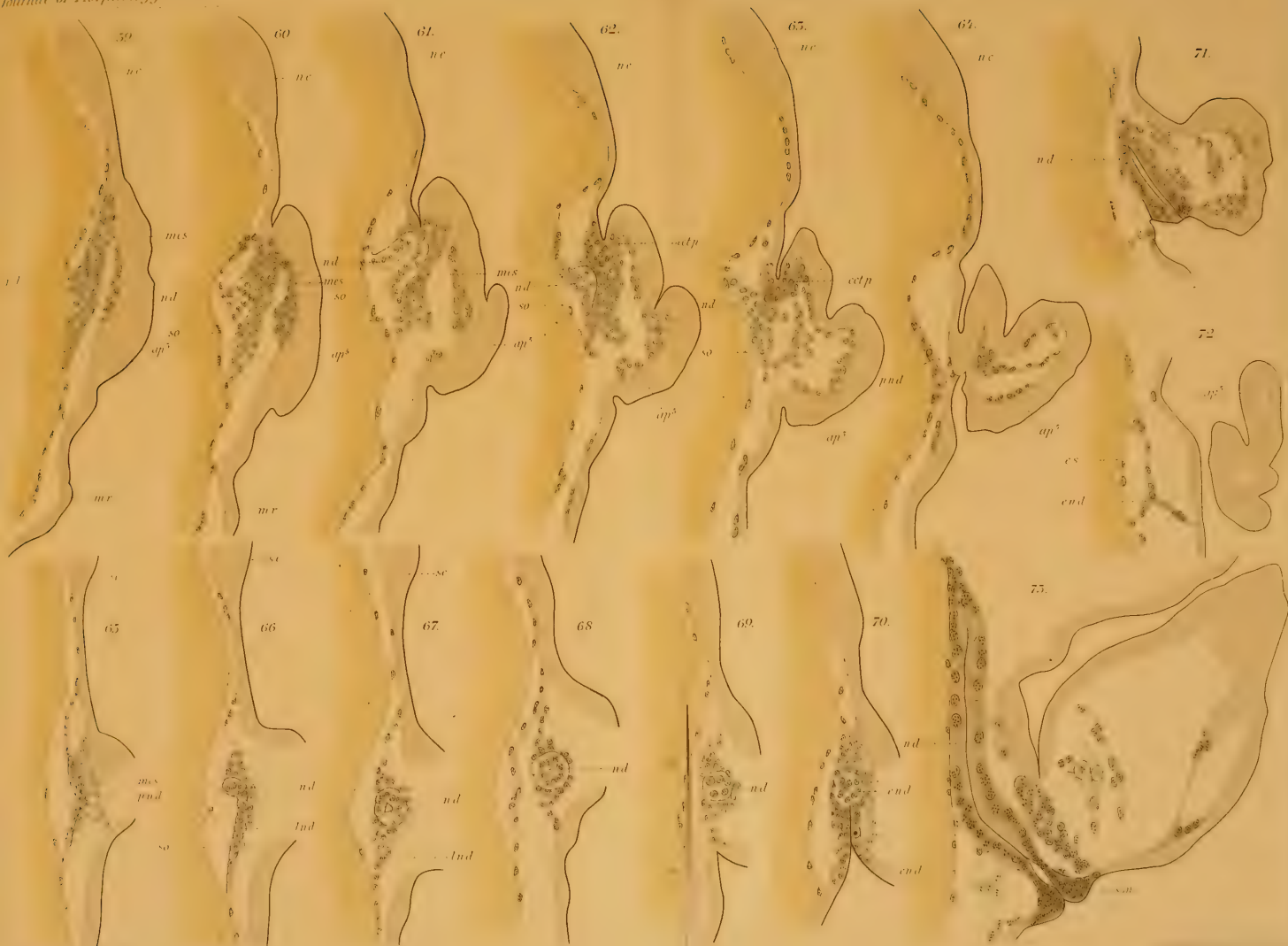
FIG. 69. Section No. 10, showing the distal end of the duct.  $\times 200$ .

FIG. 70. Section No. 11, showing the union of the ectodermic invagination with the mesodermic portion of the duct.  $\times 200$ .

FIG. 71 is a longitudinal section through the middle of the fifth appendage in the early Trilobite stage, showing the opening of the nephric duct to the exterior.  $\times 200$ .

FIG. 72. Section on the lateral side of the base of the fifth appendage in a late Trilobite stage. It shows the end sac, which extends beyond the base of the appendage on the surface of the yolk. Granular cells (*g.c.*) are developing on its ventral margin.  $\times 200$ .

FIG. 73. Longitudinal section through the fifth appendage of a specimen somewhat older than that of the preceding figures. It shows the distal arm of the nephridial duct and its opening at the posterior side of the appendage. The ectodermic portion is characterized by numerous small cells, which at the very end seem to be forming a sphincter muscle around the opening. The mesoderm cells are large, with a faintly colored protoplasm. In many specimens a lumen was continuous throughout the mesodermic and ectodermic portions.  $\times 300$ .















INDEX LETTERS TO PLATE XXVIII.

<i>c.n.t.</i> = cross-section through a nephridial tubule.	<i>l.r.c.</i> = large red cells.
<i>c.t.</i> = connecting tubes.	<i>mus.</i> = muscle.
<i>ec.p.</i> = ectodermic part of nephric duct.	<i>n.l.<sup>1-4</sup></i> = nephridial lobes.
<i>e.s.</i> = end sac.	<i>p.a.</i> = pedal artery.
<i>e.s.t.</i> = tubule of the end sac.	<i>p.l.</i> = plastron.
<i>ex.o.</i> = external opening of the nephridial duct.	<i>p.n.</i> = pedal nerves.
<i>g.c.</i> = granular cells.	<i>p.o.</i> = pockets in the walls of the duct.
<i>h.c.t.</i> = hollow cells forming tubules.	<i>r.c.</i> = red cells.
	<i>v.c.</i> = vacuolated cells.
	<i>s.r.c.</i> = small red cells.

EXPLANATION OF PLATE XXVIII.

FIG. 74. A few characteristic nephridial cells of the Trilobite stage. *a.* Longitudinal section through a cell with finely granular protoplasm around the periphery. As the section passes near the surface of one end of the cell the granules show a reticulated arrangement. *b.* A cross-section through two cells similar to *a.* The nucleus adheres to the side of the cell. *c.* Shows a small red cell on the surface of *b.* *d.* Red cells, with a clear protoplasm which took a deep stain in Lyon's blue.  $\times 475$ .

FIG. 75. Sections through the nephridial cells from one of the older specimens of the second larval stage. *a.* Longitudinal section through a tubule. *b.* Cross-section through a tubule. *c* and *d.* Cells in which large, dense-looking granules cover the nucleus.  $\times 475$ .

FIG. 76. Cross-section through the region on the dorsal side of the heart of *Limulus* in the second larval stage, showing hollow cells similar to those in the nephridial lobes. Several cells have united end to end to form branching tubules. Other cells are present filled with large granules (*g.c.*), while others of the same size have only a few granules in them.  $\times 300$ .

FIG. 77. Section through a granular cell of a young *Limulus* three-fourths of an inch long. (See Pl. XXIII, Fig. 48.) It shows one enormous granule in the center with smaller ones around it.  $\times 475$ .

FIG. 78. Section through nephridial cells, from the younger specimens of the second larval stage. *a.* Longitudinal section through tubules which show a granular periphery. *b.* Section through two cells which have united. *c.* Cross-section through a tubule. *d.* A small cell with the nucleus surrounded by granules.  $\times 475$ .

FIG. 79. Section of cells in the sixth leg of a specimen in the second larval stage.  $\times 475$ . *a.* Long hollow cells uniting, similar to those in the nephridial and pericardial regions of the same age. *b.* Large triangular cells uniting.

*c.* Cross-section through a hollow cell. *d.* Section through a granular cell. *e.* Section through a triangular cell filled with granules.

FIG. 80. Cross-section through the end sac of a specimen in the Trilobite stage. The section is posterior to the point where the nephridial duct opens into the end sac. The end sac is lined with small and finely granular cells. One large cell is shown on the dorsal side of the sac, filled with small granules, and with pseudopodia extending out from its free margins. At the lateral side the end sac shows a projection similar to both the wall of the end sac, and to the long hollow cells which are characteristic of this age. Ventral to this sac is another of similar structure, which unites with the end sac in the second section posterior to this. These projections are either outgrowths from the wall of the end sac, or nephridial tubules united with it. Scattered among the nephridial cells were a number of small red cells. Some of them had a faintly granular protoplasm, others were vacuolated, and still others in which nothing but the cell walls could be distinguished outside the nucleus.  $\times 400$ .

FIG. 81. Drawing of the injected nephric duct of an adult *Limulus* from the dorsal side. The main part of the duct is coiled and folded upon itself many times, the distal arm alone remaining straight, running from a point in front of the anterior transverse process of the plastron along the edge of the plastron as far as the fourth nephric lobe. It then passes between the muscles through the median end of the last nephridial lobe to the exterior. Along the free margin of the duct, slight projections, or pockets (*po.*), are found. In other places small connecting tubes (*c.t.*) unite different portions of the duct.

FIG. 82. In this case the duct has been dissected apart along its entire length. In many places small tubes were found, connecting one fold of the duct with another which lay either beneath or beside it. In most cases these connecting tubes had to be cut in order to free and unfold the duct. A few of them are left untouched (*c.n.t.*). In other places small evaginations or pockets in the wall of the duct were found along its free margin (*po.*). The nephric duct lies below the outer edge of the plastron, deeply imbedded in muscle, the genital organs, and in the hepatic caeca. The fourth lobe of the Nephridia shows from below.

FIG. 83. Drawing of the nephric region of an adult *Limulus*, showing the nephric duct, nephridial lobes, and the blood vessels and nerves.

The four nephridial lobes lie at the base of the second, third, fourth, and fifth legs. They are connected along their median dorsal ends by a band of collecting tubes — the stolon. A short distance from the oral ring small branches arise from the pedal arteries of the second, third, fourth, and fifth legs, and pass along the ventral surface of each of the nephridial lobes, supplying the muscles beyond. During its course, each artery sends off alternate branches to the muscles and to the lobe. The arteries which pass to the lobes break up into small branches, which fill the nephridia with a network of vessels.

Large integumentary nerves (*int.n.*), arising from the haemal side of the brain, pass out between each nephridial lobe to the sides of the carapace. On either side of each lobe a smaller nerve arises from the haemal side of the pedal nerve which supplies the tergo-coxal and the plastro-coxal muscles on the posterior and anterior sides of the base of the coxite. The nephric duct lies near the lateral dorsal side of the lobes. The distal arm passes through the posterior median end of the fourth lobe to the external opening at the base of the fifth leg. The ectodermic portion of the duct extends from the external opening to the fourth lobe.





















# THE EMBRYOLOGY OF A TERMITE.<sup>1</sup>

*Eutermes (Rippertii?)*.

## FIRST PAPER.

(Including a contribution to the discussion as to the primitive type of development, and the origin of embryonic membranes (amnion), and of the mesoderm, in the Insecta.)

HENRY McELDERRY KNOWER.

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## INTRODUCTION.

THOUGH the Termites must be ranked among those insects which have best preserved ancestral morphological traits, their development has not been studied up to the present time. Indeed, it is only within the last few years that we have been enabled to judge of the details of development of any of the primitive forms of insects. The technical difficulties which the investigator of these forms must meet are very great, much time being required to obtain few results, especially in dealing

<sup>1</sup> This paper was accepted as a thesis for the degree of Doctor of Philosophy in the Johns Hopkins University, May, 1896. (See explanatory note at the end.)

with early stages. As Wheeler (26) has suggested, no doubt this has deterred many from working on such material.

Having had the good fortune to secure Termite eggs in ample quantities, I have devoted considerable attention to the development of these interesting insects, in the hope that such a study might throw light on some vexed questions of insect embryology. The present paper must be confined chiefly to the earlier stages of development, the later changes being left to be described at another time in the near future. The eggs were obtained in Jamaica at different times, by myself, and through the kindness of Dr. Hough, Dr. Sigerfoos, and the late Dr. Conant. I am also indebted to Mr. Taylor, of Jamaica, for assistance in obtaining material, and to Dr. William Patten for valuable suggestions as to technique. It is a great pleasure to express my appreciation of the constant kindness and useful criticism and advice of Professor W. K. Brooks, during my work at the Johns Hopkins University.

#### TECHNIQUE.

My material was fixed with hot water, with cold picro-sulphuric acid (alcoholic), and with hot alcoholic picro-sulphuric, the latter giving the most satisfactory histological results when the acid was promptly washed out after fixation. Specimens fixed in hot water and transferred into 70 per cent alcohol are quite satisfactory.

As Wheeler found in working with Orthoptera, sections through these eggs are very difficult to obtain. Though tedious, Heider's (13) method of painting each section before cutting gives good results. A modification of Dr. William Patten's ingenious method of orienting small objects for cutting is most satisfactory in sectioning these eggs. I feel that my success in handling this material is largely attributable to Dr. Patten's suggestions as to technique and accuracy in methods of study.

In sectioning early stages of the Termite germ-disc, I break off most of the yolk in clove oil, with very sharp needles, under the dissecting microscope, and stick the particle to be sectioned

on a piece of tracing paper smeared with a thin layer of collo-dion fixative, and ruled with parallel lines by a needle point. The paper is now dipped into xylol and then placed in the paraffine bath. After imbedding, the paper may be stripped off, leaving lines on the surface of the paraffine which give the proper direction for cutting. It is possible in this way to obtain especially thin sections of quite early discs, in any desired plane. Enough yolk adheres to the disc, when dissected off, to make plain its relations to the interior of the egg. In later stages the entire egg is satisfactorily sectioned by the same method, care being taken to prick the side which will lie uppermost in the paraffine bath, to insure penetration. To prevent the yolk from becoming brittle, eggs which are to be sectioned should not be kept in clove oil any longer than is necessary to clear thoroughly.

In studying the egg as an entire transparent object, I have found it best to prick each specimen with a sharp needle under the dissecting microscope; then stain in Grenacher's borax carmine for two days; wash out in cold 70 per cent alcohol, acidified with 20 drops of nitric acid to every 100 cc. alcohol, for three or four days or longer, until the stain remains only in the nuclei; transfer gradually to absolute alcohol, and finally into xylol, which gives whiter and clearer specimens than clove oil.

Eggs for sectioning are usually stained in this way before cutting, though it is best not to wash these out so much, and as I have said, clove oil is then used to clear.

Good surface views of the germ-band at various ages are also obtained by staining the specimen, dissected off from the yolk, in rather strong Delafield's haematoxylin for a very short time.

#### GENERAL DESCRIPTION OF THE EGG.

There are no special chambers for nurseries, or for the queen, in the nests of this species of *Termes*. (This species has been described from Jamaica as *Eutermes Rippertii*. I shall question this for reasons which will appear at another time. Hence the interrogation mark after *Rippertii* in the title.) On cutting

open a nest, the passages which traverse it in all directions are seen to have no regular arrangement. The walls of the inner passages are thinner than those at the surface. The queen is generally found in the interior of the nest, near its base, surrounded by numerous workers and larvae, and not far from the eggs. The eggs have been collected into heaps, by the workers, as they were laid, and piled up without any apparent system in the passages near the queen. The older larvae also assist in caring for the eggs. It is an easy matter to collect quantities of eggs, since they are quite moist and adhere together in masses, in which all stages of development are to be found, from unsegmented ova to larvae emerging from the egg-membranes. Considerable time must be subsequently devoted to picking out stages from such mixed material.

The unsegmented egg is about 0.5 mm. long by 0.22 mm. in the shorter diameter. It is elongated, larger at one end, and markedly convex on one side. This shape makes it easy to determine the planes of symmetry from the start, since the enlarged or micropylar end is found to be the posterior pole, while the convex side is the ventral surface. In the course of its development the embryo changes its position by a remarkable process of "revolution," like that described for the Libellulids and certain primitive Orthoptera. This must be kept in mind in speaking of the anterior and posterior poles, and of the dorsal and ventral surfaces. As used above, the micropylar end of the egg is the definitive, as well as the primary, posterior pole, and the convex micropylar side becomes the final ventral surface.

It will appear that the embryonic rudiment when first established, and until shortly after the closure of the amniotic cavity, lies entirely on the convex ventral surface of the yolk. At this period the entire embryo occupies but a small area on this surface, just beneath the micropyles, at its extreme posterior limit, the hind end of the germ-band reaching the posterior pole of the egg.

In the last stages of development the same relative position, lost during intermediate changes, is reestablished by "revolution," so that the posterior end of the embryo comes again to



lie on the convex surface at the micropylar pole of the egg, while the head coincides with the anterior limit of the same surface.

Dr. Hagen (12) described the micropyles of the eggs of this species as follows: "The micropyles of *Termes* eggs have not before been known. Near the upper pole of the egg, on each side, there are four to six flat impressions; viewed in profile they are similar to a soup dish. In the middle of this shallow funnel is a tube of smaller diameter going through the yolk-membrane in the direction of the egg pole."

This description must be modified somewhat; since I have found that the funnels are more numerous, and are grouped on the ventral surface. The chorion is the only membrane penetrated by these funnels. The micropyles are arranged in a semicircle, on the convex face of the egg, near the posterior end. This semicircle is composed of from 12 to 18 funnels, which vary considerably in arrangement. They may be strung out into a single line, curving over the ventral surface, just above the place where the embryonic disc first appears, and extending up on either side toward the dorsal surface. Often the funnels at the two ends of the line are crowded together, while the ventral median ones form a single row (Pl. XXIX, Fig. 1). In some eggs, on the other hand, the funnels of the median ventral row are gathered together, while the lateral openings extend in a line on either side. In surface preparations the rims of the funnels at the mouths may be seen to be slightly corrugated.

Sections of the chorion above the germ-disc, as represented in Pl. XXXI, Fig. 31, cut the micropyles through the wide outer opening, the penetrating canal, or the inner opening.

Dr. Hagen (12) thought he could "see distinctly a bunch of filiform spermatozoa inside the micropyles in some eggs," and that in one case he had succeeded in bringing them out by pressure. I have never found any spermatozoa. Filiform bunches are often seen beneath the chorion. On examination such appearances have proved to be due to various objects, but never to spermatozoa. In surface views of alcoholic specimens, the folds of the viteline membrane and protoplasmic threads

attached to the chorion, at times might be mistaken for bunches of filiform bodies. When such specimens are crushed, the membrane wrinkles up and looks like bunches of filaments. The eggs from one nest had been attacked by fungus to such an extent that the hyphae, entering the micropyles, had ramified in the yolk and used it up, destroying the embryos. Dr. Hagen could hardly have confused such large objects as these hyphae with spermatozoa. As sperm failed to appear in any of my stained specimens, I am inclined to think that folds in the membranes or strands of protoplasm were mistaken for spermatozoa.

#### THE YOLK.

My observations on the yolk were made on preserved material. The yolk-mass, thus studied, is composed of a lot of polygonal bodies, which are vesicles containing a homogeneous coagulable fluid. These bodies stain deeply in haematoxylin and carmine, and very considerably in size and shape (Pl. XXXI, Figs. 30-37, *pl. b.*). The other constituent of the yolk-mass is an oily fluid distributed in small globules throughout the egg, and often found collected in one or two quite large drops. When alcoholic specimens are crushed, the oil globules flow together and run out, leaving the yolk-bodies for the most part intact. In such cases the character of the vesicles can be made out. They are found to vary in structure, part of them being filled entirely with homogeneous coagulable substance quite elastic under pressure.

Many of the bodies, however, differ from this. They contain the homogeneous stainable substance, but also little oily drops, of which there may be only a few or a great number in a vesicle. The greater the collection of these drops, the less is the homogeneous material, so that it is often reduced to a porous substratum for the drops. It is possible to crush such vesicles so that the oil drops escape through the membranous walls. Examined in clove oil or in sections the yolk has quite a different appearance. In these specimens the oily matter is all dissolved out of the yolk, even from the interior of some of the vesicles. Treatment with oils and subsequent heating dur-



ing imbedding often cause numbers of the yolk-bodies to fuse together into large bodies. This may be very marked, large spaces being left in the yolk by the solution of the oily fluids. In sections single yolk-bodies are frequently found riddled with holes, left by the solution of the oil drops formerly imbedded in their substance (Pl. XXXI, Figs. 30, 31, 34, 37, *p. pb.*).

I should derive the yolk-bodies in which oil drops have collected from the homogeneous vesicles. The homogeneous (albuminous) coagulable fluid of these bodies apparently becomes transformed into soluble substances which, first forming in isolated drops, finally fill the whole vesicle. In this way the oil globules and free oily fluids of the yolk would appear to arise from the (albuminous?) yolk-bodies, to furnish the growing embryo easily assimilable nutriment. The sections (Pl. XXXI, Figs. 30, 33, and 34) show finely fragmented yolk-bodies lying beneath the embryonic disc. Other sections do not show a similar fragmentation, the solution having been more complete. When yolk-bodies in which drops have collected are prepared for sectioning by the usual methods, chemical changes take place which result in homogeneous fused masses of stainable (albuminous?) substance, and in the solution and removal of the fatty matters.

There is apparently no definite arrangement of the different yolk elements. There is no peripheral layer of protoplasm distinguishable before the formation of a blastoderm. I have found no "segmentation of the yolk" during the early stages of development.

#### SEGMENTATION AND THE CHANGES IN THE BLASTODERM LEADING TO THE ESTABLISHMENT OF THE FIRST RUDIMENT OF THE EMBRYO.

The first sharply marked rudiment of the embryo is, as in the case of certain of the Orthoptera (*Stenobothrus*, *Stagmomantis*, *Gryllus*, and *Oecanthus*), a relatively small disc of closely crowded cells at one pole of the egg. Since the history of the origin of these embryonic discs has not been worked out, I have studied, with special care, the segmentation

and those changes in the blastoderm cells which result in the formation of the embryonic rudiment on the surface of the yolk.

I have endeavored to answer the following questions: Is the disc formed immediately during the segmentation, by cells wandering directly to the point on the surface where the disc is to appear? Is a blastoderm over the entire yolk surface first formed as a result of segmentation, and then the disc from its cells? Again, if this latter is the method, is the embryo a result of a simple multiplication of the cells of a restricted area of the blastoderm, or is there some other factor present in the formation of the disc?

The answer to these questions requires considerable attention to the earliest stages, but is of much importance to an understanding of the origin of the "under-layer" and of the amnion.

The position of the polar-bodies marks the dorsal pole of the shorter axis of the egg. I have not studied the formation of the polar-bodies from the nucleus, as the few eggs which were at the proper stage did not show the process distinctly enough. To separate from the polar-bodies, the segmentation nucleus moves from the center of the yolk to the center of the dorsal surface. After their formation, the polar-bodies appear as two little rod-like masses of chromatin surrounded by a small quantity of protoplasm, and lie at about the middle point of the flattened dorsal surface of the yolk-mass (Pl. XXIX, Figs. 2 and 3). Later the chromatin breaks into fragments, but the little collection remains visible for a number of divisions.

The segmentation nucleus, on returning from the dorsal surface, lies, just previous to the first division, in the center of the yolk at the intersection of the shorter and longer axes of the egg (Pl. XXIX, Fig. 2).

The first spindle lies at right angles to the shorter axis, so that one of the cells arising from the first division wanders toward the enlarged posterior pole, where the embryo will first appear. The other cell remains near the position that the mother nucleus held (Pl. XXIX, Fig. 3). At the start, then, there is a decided proliferation toward the future embryonic area.











This is brought out better in the following stage, which exhibits two cells in the enlarged end, one on the shorter axis, and one in the small end; that is, there are three nuclei nearer the posterior than the anterior pole (Pl. XXIX, Fig. 4). The cleavage becomes irregular with the eight-cell stage, one or more nuclei dividing before the time for a typical rhythm of divisions. For several divisions there is a slight preponderance of cells in the larger end of the egg. For instance, one egg has four nuclei in this end, one on the shorter axis, and three anteriorly; while another has five in the posterior, and four in the other end (Pl. XXIX, Fig. 5). Generally, during the early stages of cleavage, there are three or four more cells in the larger than in the smaller end of the egg. After five or six divisions, the resulting nuclei have taken positions at about equal distances apart through the yolk. The nuclei are each surrounded by a little mass of protoplasm, and may consequently be spoken of as cells. As far as can be determined, there is no protoplasmic continuity between these cells at this early period. Later, when the embryonic disc begins to appear, continuity is established between its cells; but even then a connection between the blastoderm cells of other regions, or between these and the yolk-cells, is not made out with any degree of certainty. A view of the ventral surface of an egg at this stage shows very well the equal distribution of the nuclei on that side, and the same is found to be true of the nuclei on the remaining surface of this egg (Pl. XXIX, Fig. 6). (*Refer to end of paper, to the explanation of Figs. 4 and 5, in regard to cleavage.*)

Most of the cells have now reached the surface, there being only a few in the yolk which lie at equal distances apart. In properly prepared material, the changes that follow and lead to the appearance of the embryonic disc can be most distinctly traced in entire, transparent eggs studied in clove oil, cedar oil, and balsam. The following description refers chiefly to specimens studied in this way and to sections through certain stages. I have already stated that the various stages are mixed together indiscriminately when collected. The series illustrating the growth of the disc had to be picked out from a great mass of

material. There can be little doubt, however, that a typical series is here figured, for the figures are based on an examination of a great many specimens, and the chief stages are well marked.

Since the first rudiment of the embryo is formed from surface cells alone, the few yolk-cells may be neglected in the description.

Pl. XXIX, Figs. 7-10<sup>a</sup>, represent successive changes on the surface of older eggs. The nuclei are found at all points on the surface in the act of dividing, or in pairs just subsequent to division. In the posterior half of the egg this activity becomes especially pronounced, while the nuclei of the anterior half are comparatively inert.

Three surfaces of a somewhat older egg are shown in Pl. XXIX and Pl. XXX, Figs. 11-11<sup>b</sup> (ventral, dorsal, and lateral views). As compared with the preceding figures and with the following ones, it is evident that the number of cells in the anterior half of this egg has reached a maximum, which remains constantly about the same in older specimens. The nuclei in this half are few and widely separated. The opposite end, on the other hand, is the seat of active multiplication and change. This is true of the whole posterior end, but it is evident in the three views of the egg before us that the dorsal (Pl. XXX, Fig. 11<sup>a</sup>) and lateral (Pl. XXX, Fig. 11<sup>b</sup>) surfaces of this half are less crowded with nuclei than is the ventral side. The ventral surface (Pl. XXIX, Fig. 11) exhibits an extensive area of rather closely crowded nuclei, stretching to the extreme limits of the surface posteriorly and laterally. A side view (Pl. XXX, Fig. 11<sup>b</sup>) shows a considerable lateral extension of this area, relatively crowded as compared with the rest of the surface.

The posterior half of the surface represented in Pl. XXIX, Fig. 10, exhibits an activity in division and a distribution of nuclei of about the same intensity in its entire extent, forward to the shorter diameter of the egg. A line drawn through the shorter diameter of this figure divides rather sharply an anterior half, with but few widely separated nuclei, from a posterior half, in which the nuclei are more numerous and lie comparatively close together down to the line just drawn. Near the

posterior pole this area is slightly more crowded than near the shorter diameter; but there is very evident activity here, contrasting sharply with the inertia of the cells on the anterior side of the line.

Drawing a similar line across the middle of the older egg (Pl. XXIX, Fig. 11), we find no change anterior to the line. In the region just posterior to this line, extending as far back toward the pole as a second line drawn parallel through the anterior end of the dotted pointer *ca.*, there are fewer nuclei than in a corresponding region of the younger egg (Pl. XXIX, Fig. 10) — by actual count, nearly one-third less than in the earlier stage, or 26 to 36 nuclei. On the other hand, in area *ca.*, Pl. XXIX, Fig. 11, a decided increase in the number of cells is evident, as compared with the preceding stage. The nuclei here are not only one-third more numerous (about 101 to 157), but are much more closely crowded together.

Such a comparison indicates strongly that, in addition to a special activity in cell division within the area *ca.* of Pl. XXIX, Fig. 11, certain cells have actually wandered into this area from more anterior portions of the surface.

If the number of cells in the region anterior to *ca.*, down to the line through the shorter diameter, had remained the same as in the preceding younger stage (Pl. XXIX, Fig. 10), there would have been reason to conclude that this constant number had been maintained, in spite of a multiplication of cells, by a migration back into *ca.* One-half of the product of the divisions of the nuclei might have wandered back into *ca.* from the more anterior region, without disturbing the relations existing in Pl. XXIX, Fig. 10. As it is, the evidence of a migration back into the area *ca.* is much stronger, since an actual decrease in the number of nuclei anterior to *ca.* has been shown; while the increase in the cells of *ca.* is sufficient to allow for this addition from without, as well as for that from a multiplication of the cells already within its limits.

Similar results are obtained from a comparison of dorsal surfaces.

It may be claimed that this method is inconclusive, since the specimen from which Pl. XXIX, Fig. 11, was drawn cannot be

proved to have certainly passed through a stage like that of Pl. XXIX, Fig. 10, having been selected from a lot of eggs in which all stages were mixed indiscriminately. The condition shown in Pl. XXIX, Fig. 11, may have been reached without migration by a more active multiplication of cells in the area *ca.* from the first, the blastoderm anterior to this region remaining comparatively inert. In other words, the center of activity may have been placed more anteriorly in Pl. XXIX, Fig. 10, than in Pl. XXIX, Fig. 11, from the start.

In spite of this possibility of error, I believe the figures do represent successive stages, and that the area *ca.* on the surface of the egg in Pl. XXIX, Fig. 11, etc., has been established, not only by a multiplication in that region, but also by the addition of cells migrating into it from without. This conclusion seems justified by a similar examination and comparison of many eggs in these stages.

Pl. XXX, Fig. 12, is a slightly older ventral surface showing a like extension of the area *ca.*, where more nuclei are now found. Note especially the rather short intervals between the nuclei in the posterior and lateral regions.

Pl. XXX, Figs. 13 and 14, exhibit in ventral and lateral views a further result of the processes just studied.

Comparing Pl. XXX, Fig. 13, with the younger stages in Pl. XXIX, Fig. 11, and Pl. XXX, Fig. 12, the number of nuclei in regions anterior to the area *ca.* is seen to have remained constant, in spite of a multiplication of cells there being demonstrable. Within the former area *ca.* there has been a great increase of nuclei, especially near the center. This is undoubtedly due in part to continued cell division here; but also, as the above observation makes plain, there is evidence of an addition of migrating cells resulting from multiplication in more anterior regions.

Comparing Pl. XXX, Figs. 13 and 14, still closer with Pl. XXX, Fig. 12, additional and striking evidence is found of a further migration of cells from the boundaries toward the center of the former area *ca.*

On rolling the egg figured in Pl. XXX, Fig. 12, the area *ca.* stands out more sharply from the surrounding surface than is



shown in the figure. Near the lateral and posterior boundaries, as well as in the center, the nuclei are about equally distributed and lie rather close together. Turning to the older stage (Pl. XXX, Fig. 13), it is evident that the nuclei in the lateral portions of the same area are fewer than in the younger egg, and nearly twice the distance apart. The egg (Pl. XXX, Fig. 14), being rolled slightly on one side (though not nearly so much so as Pl. XXX, Fig. 11<sup>b</sup>, with which it must not be compared), shows this better than Pl. XXX, Fig. 13, in which the convexity of the surface makes it impossible to give an accurate idea of the distribution of the nuclei at the sides. The letters *l.b.d.* indicate a like region in both figures (Pl. XXX, Figs. 13 and 14). It is the portion of the surface lying outside of (lateral to) the position marked by these letters that shows a diminution in the number and a wider separation of the nuclei, as compared with the previous stage.

These changes within the limits of the posterior half of the ventral surface, between the stages of Pl. XXX, Figs. 12 and 13, resulting in a perceptible diminution in the number of nuclei laterally, with an increased crowding toward the center, apparently necessitate an active migration of cells centripetally, coöperating with cell multiplication, to establish the embryonic disc.

Pl. XXX, Fig. 15, is an example of an older egg, showing an extreme concentration of the embryonic disc.

In Pl. XXX, Fig. 18, which represents the ventral surface at a much later stage, the embryonic region, now appearing as a conspicuous and sharply defined circular disc of nucleated protoplasm, hardly occupies one-half of the area formerly marked *ca*. The surrounding cells are few and widely scattered, while the comparatively broad, crowded area in the earlier figures (Pl. XXX, Figs. 12 and 13) has contracted to the smaller, densely crowded, circular embryonic rudiment. There is a marked concentration in the germ-disc visible in passing from the stage shown in Pl. XXX, Fig. 17, to that of Pl. XXX, Fig. 18. Note the concentric crowding of the nuclei along the sides of the disc in Pl. XXX, Fig. 18, as compared with the preceding figure.

A study of sections of eggs passing through these stages apparently confirms what is learned from surface views.

In its early stages the embryonic disc is in cross-section a comparatively broad, flat plate of protoplasm formed by the fusion of its cells, the neighboring cells of the blastoderm being connected rather loosely with the edges of this area (Pl. XXXI, Fig. 30). In reaching its final restricted size in Pl. XXX, Fig. 18, the broad plate of protoplasm, whose boundaries were well defined in an earlier section, has become much reduced in extent. The section of the completed disc (Pl. XXXI, Fig. 31) shows the plate contracted to a decidedly shorter diameter. (The two sections (Pl. XXXI, Figs. 30 and 31) are drawn to the same scale.)

The manner in which the mesoderm arises (described further on), partly by a crowding of cells below from the embryonic area as it becomes defined, is another argument in support of the view here advanced for the formation of the first rudiment of the embryo.

The area of the blastoderm, the origin and gradual concentration of which we have thus traced, will be henceforth spoken of as the embryonic area or germ-disc. Though it might be so called at an earlier stage, it hardly merits the term before reaching the definiteness of outline shown in Pl. XXX, Fig. 18.

The facts here reviewed appear to me to prove that the embryonic disc is not formed directly in the segmentation by cells wandering toward a predetermined point. The evidence indicates also that the disc is not the result of simply active cell multiplication in a restricted area of the blastoderm. The truth seems to be that segmentation results in the establishment of a blastoderm of cells scattered over the entire surface of the yolk, and that then, as these cells increase in numbers, a process of concentration draws many of them together to form an area on the ventral surface, which is the first rudiment of the embryo, the germ-disc. This is shown in the entire series of stages figured, and is brought out vividly by a comparison of Pl. XXX, Figs. 12 and 13, with Pl. XXX, Fig. 18. In Pl. XXX, Fig. 12, the embryonic area spreads over the whole of the posterior half of the ventral surface of the yolk. In Pl. XXX,



Fig. 13, the limits of this diminishing area have drawn well in toward the center and away from the lateral margins of this portion of the ventral surface. In Pl. XXX, Fig. 18, the germ-disc hardly covers one-half of its extent in Pl. XXX, Figs. 12 or 13.

The appearances are not at all what would be expected from a simple cell multiplication in a restricted area. In such a case the growing disc should, it seems, be formed from the coalescence of several areas multiplying around separate centers, or should spread out on all sides as its cells multiply around a single center. As the figures show, the disc is here formed by a steady contraction of a primarily extensive area toward a central point.

The fact that, at even so late a stage as one showing the amnio-serosal fold, the nuclei of the disc are of the same size as those in the surrounding blastoderm, perhaps lends some support to the above contention; since we should expect a rapid multiplication within a restricted area of the blastoderm to produce a mass of cells in that region of smaller size than on the surface elsewhere. In the Termite, during this period, the nuclei of the blastoderm in the whole posterior half of the egg appear to divide with about the same rapidity. The process of concentration, which draws the cells together to form the disc, is accompanied by a steady multiplication of the cells about to be incorporated in it, but the nuclei of the rest of the blastoderm divide also. The position of the embryonic disc is consequently not marked by nuclei smaller than those elsewhere on the blastoderm, in the stages we are considering.

The first rudiment of the embryo is certainly not formed around a number of discrete centers, as is claimed for some decapod crustacea and certain insects. The concentration leading to its first formation is, from the start, most apparent in the posterior portion of the disc. The posterior border becomes sharply defined at an early stage, as the cells draw together in concentric rows from the posterior pole. The lateral edges are next involved; but much later, when the disc is otherwise well outlined and its cells are quite closely crowded, the nuclei of the anterior end have not yet drawn together (Pl. XXX, Figs. 18 and

19). When the amnion is about to close over, the cells of this end have drawn together and become incorporated in the disc.

I cannot determine whether the concentration, in the early stages, is accomplished by the migration of independent amoeboid cells toward the embryonic area, or whether the blastoderm outside this area is from the first a continuous membrane of loosely connected cells which contracts toward the center of the germ-disc. I believe, however, the blastoderm cells beyond its limits to be independent, to a late stage in the formation of the disc.

A less marked concentration of the surface cells has been observed in other insects in similar stages, resulting in a closer approximation of the cells of the embryonic area. Refer to Patten (21), Figs. 1 and 2 of Pl. XXXVI (A), and Fig. 5 of Pl. XXXVI (B), and Wheeler (25), Figs. 63, 64, 66, and 68.

In the Termite's egg, where the embryo is a comparatively small disc when completely established, the concentration to establish this disc is an especially notable process.

McMurrich (18) has discovered a similar method of the formation of the embryonic rudiment in Isopods. His figures, 17-19 and 50-52, show the formation of the germ-band in these crustacea by a concentration of the surface cells toward the ventral side of the egg. He finds an intimate connection between this phenomenon and the formation of an "under-layer," and my observations on the Termite's egg lead me to a similar conclusion for it. Hence the detail in which I have described the early stages.

#### ORIGIN OF THE MESODERM.

I have studied the origin of the under-layer with especial care, on account of the recent conflicting results of Wheeler (26) and Heymons (14) in regard to its formation in the Orthoptera.

In the Termite there is no gastrula invagination. The under-layer begins to appear at an early stage in the formation of the disc, somewhat earlier than Pl. XXX, Fig. 14, when its cells first begin to be crowded. During this period, at irregular points in the embryonic area, lateral as well as median,

some of the cells are pushed below the surface by the concentration of the blastoderm. Other cells are separated toward the under surface of the ectoderm, by tangential divisions of its nuclei, at various scattered points (Pl. XXXI, Fig. 30).

As these processes continue, the under-layer constantly gains in bulk. Its formation is to be traced back to the concentration of the cells of the disc, and when this has reached the stage represented by Pl. XXX, Fig. 18, the under-layer cells have for the most part collected into a plug projecting into the yolk. From the surface this plug appears as a darkened area of crowded nuclei near the center of the disc.

Preparations of a series of discs, after the under-layer has become thus crowded into a plug, illustrate the growth of this collection of cells. Pl. XXX, Figs. 16-19<sup>a</sup>, show, in surface views, the gradual extension of the plug, up to the time when the amnio-serosal fold has grown well forward over the disc.

Sections through these stages and those just preceding and immediately following, taken in connection with what has been learned from surface views, give interesting data as to the formation of the under-layer and the amnion.

Pl. XXXI, Fig. 30, gives a cross-section of the single-layered disc at a stage somewhat older than Pl. XXX, Fig. 13, when it is first definitely outlined from the surrounding blastoderm. There is a crowded appearance of the cells, and some of the nuclei are displaced from the surface and seen wedged below. At various points in the surface layer, at the sides as well as near the middle, nuclei are also found in the act of dividing toward the lower surface, thus adding to the number of cells adhering in the lower layer of the disc.

A cross-section (Pl. XXXI, Fig. 31) of the embryonic area through the region of the plug at the stage (Pl. XXX, Fig. 18), when compared with Pl. XXXI, Fig. 30, cutting the same region of a younger disc, shows that the plug has grown considerably by the gradual addition of cells from the ectoderm and their subsequent multiplication. The mesodermal plug is still in close continuity with the ectoderm.

A sagittal section of a disc of this age (Pl. XXXI, Fig. 32) shows the plug quite distinctly.

Both surface views and sections of these stages agree in exhibiting no gastrular groove. On the contrary, it is as I have stated — the under-layer arises at all points in the germinal disc, as a result of the concentration of this area and of the tangential divisions of its cells. The formation of a mesodermic plug is apparently a further outcome of the concentration. (Consult McMurrich (18) on the formation of the under-layer in Isopods.)

*A discussion of the general bearing of these facts on the origin of the mesoderm in insects will be found further on.*

#### ORIGIN OF THE AMNIO-SEROSAL FOLD.

I have devoted much attention to the early history of the embryonic membranes, on account of the general interest their presence excites.

When the amnio-serosal fold is first clearly defined as a fold in sections, it appears from the surface (Pl. XXX, Figs. 19 and 19<sup>a</sup>) as a semilunar fold along the posterior border of the embryonic disc, extending forward on either side toward the anterior end. Sagittal sections of this stage make plain that the inner or amniotic layer of the fold is not distinguishable from the ectoderm of the germ-disc, except by its position (Pl. XXXI, Fig. 33). It is of the same thickness as the ectoderm, and its nuclei are arranged in the same layers, inverted. The outer or serosal portion of the fold, on the other hand, is quite different (Pl. XXXI, Fig. 33). This is a thin membrane of much flattened cells with nuclei far apart. This membrane resembles the rest of the extra-embryonic blastoderm of which it is a continuation. (*This evident distinction between amnion and serosa is important, as will appear further on.*)

Figs. 19 and 33 of Pls. XXX and XXXI, though representing the amnion when first appearing as a completed fold, do not exhibit the earliest stage in the formation of the amnio-serosal fold of the Termite.

Several stages before a fold can be made out in sections, its position is outlined on the surface of the disc. When the under-layer plug first appears in surface views, the embryonic disc



is quite sharply marked out, especially on its posterior border (Pl. XXX, Figs. 16 and 17). It is along this border that the amnion is to appear. Pl. XXX, Fig. 18, with the two figures just referred to, shows that, as concentration of the embryonic area proceeds, the nuclei at the posterior end draw together into the disc in concentric rows, which results in a closely crowded semicircle of cells that becomes quite conspicuous in surface views. In Pl. XXX, Fig. 18, this semicircle has become a band of nuclei, much darker than the region of the disc just in front of it, where the nuclei are not so densely crowded.

Sagittal sections of discs in these stages (Pl. XXXI, Fig. 32), younger than that illustrated by Pl. XXXI, Fig. 33, teach that the posterior margin, corresponding to the dark semicircle on the surface, differs from the rest of the disc only in a somewhat greater thickness of the ectoderm. There is as yet no fold in sections.

It is evidently the posterior thickened margin of Figs. 18 and 32, which has folded over in Figs. 19 and 33, to become the amnion.

It will be noted then, in reference to the origin of the amnion, that it is formed with the disc in the same process of concentration, and that it is, at first, evidently merely a specialized portion of the disc before folding forward to become the amnion.

This agrees essentially with the figures which Bruce (6) gave for Mantis (Pl. IV, Figs. 42 and 43); with Patten's (21) description and figures of the Phryganid; with Will's (27) account of the Aphids; and with the results of most observers, though all do not agree in regarding the amnion as a part of the embryonic rudiment.

*I have reserved a final section of this paper for a general discussion of the origin of the membranes in insects.*

#### CONTINUED GROWTH OF THE AMNIO-SEROSAL AND MESODERMAL RUDIMENTS TO THE CLOSURE OF THE AMNIOTIC CAVITY.

Preparations of eggs illustrating successive stages in the closure of the amniotic cavity show that this is accomplished by the single semilunar fold growing forward from the posterior

end of the disc. There are no separate lateral folds, nor is there any "head-fold." In a series of specimens represented in Figs. 19-24, Pls. XXX and XXXI, the membranes are found extending further and further anteriorly over the disc. In Pl. XXX, Fig. 23, the amniotic cavity remains open in only a single spot at the anterior extremity of the disc, the closure of which opening, in Pl. XXXI, Fig. 24, completes the process.

A series of sagittal sections, like that shown in Pl. XXXI, Figs. 32-35, gives a better idea of what has just been pointed out in the surface figures. (The nuclei in the resting stage in this series of figures are represented in solid black for the sake of clearness. They resemble those in Pl. XXXI, Figs. 30 and 31, being large, vesicular, and containing fragmented masses of chromatin.)

Pl. XXXI, Fig. 32, already referred to in a previous section, exhibits the appearance and relations of the amnio-serosal and mesodermal rudiments when first well established. The mesodermal collection of cells lies under the anterior half of the embryonic disc. It does not extend beneath the extreme anterior end, and is still rather intimately associated with the ectoderm from which it arose. Behind this mesodermal plug, and between it and a posterior thickening of the ectoderm (already indicated as the first rudiment of the amnion), is a thinned region of the disc with only one layer of nuclei, corresponding to the lighter portion of the surface view in a like position. Note the immensely enlarged yolk-cell nucleus as compared with one of the mesoderm.

In Pl. XXXI, Fig. 33, a section of the stage (Pl. XXX, Fig. 19), except for an increase in the size of the rather loose mesodermal plug (due partly to a continued migration from the ectoderm, as indicated by the direction of the spindle of the dividing ectoderm nucleus anteriorly, and by the crowding of the cells in the lower layers of the ectoderm), the most striking change is a bending forward of the thickening, marked amnion in the preceding stage, to form a fold. The bend takes place in the thin, single-layered portion of the disc. The serosal cell posteriorly is much flattened, and is drawn forward by a very slender thread of protoplasm. It is interesting to observe, in











this section and the following ones, fine protoplasmic processes running out from the ectoderm. In some instances I have traced such threads out to the chorion and into the micropylar funnels.

As the cells of the amniotic fold have multiplied, it has bent well forward in the next figure (Pl. XXXI, Fig. 34). Its cells form a thick mass and are arranged in two layers. Posteriorly it passes into the ectoderm through the thinned region pointed out in the former stage. The flat serosal cells lie superficially drawn forward with the amnion. The mesodermal plug is more sharply defined from the ectoderm, its cells lying loosely together in the former position and dividing in places.

When the amniotic cavity is finally closed completely (Pl. XXXI, Fig. 35), as in Pl. XXXI, Fig. 24, from the surface, the resemblance between the amnion and the ectoderm is most striking. The cells of both are arranged in two layers and divide in a similar manner. The serosa is now a very thin membrane of large, flat cells, stretching over the embryo and enclosing the yolk. Its nuclei are found, from now on, in resting condition, with one or more nucleoli and granular looking chromatin. They divide seldom. The mesoderm is now sharply separated from the ectoderm, and from this time the separation appears to be maintained. A few mesoderm cells have pushed back to the extreme posterior end of the embryo. At the anterior end the former relations remain unchanged. The yolk-cell nuclei are of remarkable size and have apparently remained undivided from an early stage.

GROWTH OF THE DISC-SHAPED EMBRYONIC RUDIMENT INTO  
AN ELONGATED GERM-BAND UP TO THE TIME  
OF ITS SEGMENTATION.

Comparing Pl. XXXI, Fig. 24, with figures of earlier stages, it is evident that considerable change has taken place in the shape of the embryo. The disc has now grown larger. It is about twice as long as broad, and while the posterior end is enlarged and rounded, the anterior extremity is rather pointed. The cells of this disc and of the amnion have become much

smaller by repeated divisions, while those of the serosa are now comparatively very large, having before this practically ceased to divide. This transparent egg also shows the few large yolk-cells, seen better in sections.

The growth of the embryo, from the time when the amniotic cavity is completely closed, is chiefly at its posterior end. The hind end of the embryonic band pushes back over the posterior end of the yolk-mass, just beneath the serosa (Pl. XXXI, Figs. 25, 27, and 29), while the head end remains fixed. (In some exceptional eggs the embryo is found out of its usual position, slipped forward or backward.) This growth continues for some time over the posterior pole, no marked change being apparent superficially, except an increase in length and breadth. The anterior end, however, becomes gradually less pointed.

A germ-band slightly older than that shown in Pl. XXXI, Fig. 24, while not yet one-half the length of that in Pl. XXXI, Fig. 26, would have already acquired a square, broad anterior end, as in the later stage.

The embryo in Pl. XXXI, Fig. 26, is not in the usual position at this period, some few eggs thus exhibiting the germ-band entirely on the ventral surface, and giving its shape and relations better than can be shown by drawing an embryo dissected-off from the yolk. Pl. XXXI, Fig. 27, represents in side view this same stage, as it is found usually, with the few exceptions just noted.

The germ-band now continues to push back around the yolk-mass, until about one-third up on the flattened dorsal side of the egg, when the embryo forms a *U*-shaped figure, lying over the enlarged end of the yolk (Pl. XXXI, Fig. 29). At this time the band is still unsegmented. Posteriorly it terminates in a rounded extremity. The anterior end has in the mean while undergone considerable change. From being a narrow-pointed tip to the band (Pl. XXXI, Fig. 24), it first gradually widened into a square end (Pl. XXXI, Fig. 26, and stages between this and Pl. XXXI, Fig. 24), and finally spread out over the yolk anteriorly and laterally, until now (Pl. XXXI, Figs. 28 and 29) this region has become the most prominent part of the embryo. Anteriorly, just in front of the point where the mouth is to appear,



the cephalic region is slightly emarginated. On either side it extends up on the yolk as a broad lobe with rounded borders. Such is the appearance of the embryo just before segmentation. (*See also next section for a description of Pl. XXXI, Fig. 28, of this stage.*)

CHANGES IN THE MESODERM AND AMNION DURING THE  
ELONGATION OF THE GERM-BAND BEFORE  
ITS SEGMENTATION.

Pl. XXXI, Fig. 36, is a sagittal section through a stage in the elongation of the embryo, slightly older than that of Pl. XXXI, Fig. 24, when the anterior end has broadened and become square, as in Pl. XXXI, Fig. 26. Compared with Pl. XXXI, Fig. 35, this whole embryo is decidedly longer. The amnion appears thinner, its cells are becoming arranged in a single layer, especially at the anterior end. As the germ-band has grown posteriorly, the mesoderm has multiplied by a division of its own cells and followed back, not quite so rapidly as the ectoderm, becoming a flattened pad of cells beneath this layer. (The mesoderm cells are well seen as a flat layer beneath the entire width of a germ-band of this age dissected-off and stained for a surface view.) The mesoderm extends no further forward than in section, Pl. XXXI, Fig. 35, but the ectoderm of the anterior end of the embryo has pushed out in front to a slight degree.

Pl. XXXI, Fig. 25, is a side view of an egg of about the same age as that sectioned in Pl. XXXI, Fig. 36. The embryo occupies a peculiar position for one of this stage, ordinarily being found on the ventral surface as shown in the younger egg (Pl. XXXI, Fig. 24). It appears to have slipped back into the exceptionally large space between the chorion and yolk. It gives a good idea of what is shown in the section, Pl. XXXI, Fig. 36, just described. Note the inflated amniotic cavity. The amnion is seen partly in optical section where it passes into the ectoderm posteriorly, and anteriorly where it is drawn out into a thin membrane. On its surface the cells form a mosaic. The mesoderm cells lie loosely beneath the

thick ectoderm and, in this case, form an especially large mass under the posterior end of the band.

Turning to Pl. XXXI, Fig. 37, we find several important changes. It is a section of the stage in Pl. XXXI, Fig. 27, before the appearance of cephalic lobes. The embryo now forms an elongated band bent over the posterior pole of the egg. The mesoderm has followed the growing posterior end and has become arranged in a thinner layer. Its anterior cells appear to have retained their primary position, as in the preceding stage, but the greater mass of mesoderm has been carried back with the elongating ectoderm, leaving only a single layer beneath the middle of the embryo. This growth of the mesoderm is, I believe, accomplished independently of the ectoderm, by a multiplication and rearrangement of its own cells. There is still a sharp division between the two layers. The growth seems to be more active at the posterior end, while the middle region appears to be pulled out, as it were, the anterior end remaining stationary. The size of the yolk-cells still precludes a later origin of entoderm from these. There is no trace of entoderm up to the time of the segmentation of the germ-band. The ectoderm just in front of the anterior limit of the mesoderm has grown further forward than in the preceding section (Pl. XXXI, Fig. 36). This anterior extension of the ectoderm will continue in later stages, and give rise to the cephalic lobes.

The effect of the backward elongation of the germ-band on the amnion, whose cells are now apparently multiplying but seldom, is well shown in the section before us. Posteriorly it still retains to a slight degree the character of the ectoderm, though much thinned out. Anteriorly the amnion has been stretched out by the pull from behind into a very thin membrane of flattened cells. I have found but few dividing nuclei in the later stages of the amnion, the membrane appearing to be stretched rather than to actively grow. This is beautifully seen in surface preparations, where the amniotic cells, now much larger than those of the more rapidly multiplying ectoderm, stand out in bold relief, lying closer together posteriorly.

The oldest stage of the germ-band just before segmentation is dissected-off from an egg like that in Pl. XXXI, Fig. 29,

and drawn in Pl. XXXI, Fig. 28. It is flattened out with the under (or yolk) surface uppermost.

This embryo exhibits a uniform ectoderm, with cells somewhat more closely crowded in the cephalic lobes. Along the borders of these expansions this crowding is greatest. At the extreme front end of the band, in the median line, wedged in between the lateral lobes, there is a small triangular area of ectoderm, in some preparations much more distinctly shown. Cells of the amnion are seen at the edges of the germ-band. The under, mesodermal layer is shown in such preparations very beautifully. Its cells being differently shaped from those of the ectoderm, lying more loosely, and at the same time staining rather more intensely, the entire layer stands out with remarkable distinctness. A larger collection under the posterior end of the band is apparent, as was shown in sections of the younger embryo (Pl. XXXI, Fig. 37). Passing anteriorly the cells become more scattered. Only two or three cells have wandered forward into the cephalic lobes — the anterior end of the mesoderm being fixed at the base of this region. Here there is a little collection, on either side, under the posterior ends of the cephalic lobes.

Graber's (9) preparations of the germ-bands of *Stenobothrus variabilis*, removed from the yolk in like manner, make a similar picture. His Fig. 76 of Taf. VI represents a stage which may be compared with my Pl. XXXI, Fig. 28, for the Termite, though the cephalic lobes are not so broad in *Stenobothrus*. In the Termite the mesoderm does not lie so evidently along the middle line, but forms a flat layer extending nearly to the edges of the band. The earlier germ-bands of the Termite have a shape somewhat different from those of *Stenobothrus* (Graber (9), Figs. 74 and 75), and here again the mesoderm is not so markedly on the middle line.

#### GENERAL SKETCH OF THE DEVELOPMENT FROM THE FIRST APPEARANCE OF SEGMENTS UP TO HATCHING.

Before proceeding to a discussion of the phenomena which have been described, I shall trace the remaining course of

development briefly, referring to the series of diagrams on Pl. XXXII for the general characteristics necessary to an understanding of this special study. A complete series of figures of the later stages will be published in the near future.

The first traces of segmentation and appendages appear, suddenly, a little later than the last stage described, where the germ-band had become a *U*-shaped cap over the posterior end of the yolk-mass (Pl. XXXI, Fig. 29). At this stage the antennae have just become evident as backward processes of the cephalic lobes, post-oral in position. The first maxillary and first thoracic are more distinct than the other anterior segments, which are however outlined. The "tail-piece" is long and unsegmented. The anterior segments through the first thoracic have therefore arisen almost simultaneously. There are no macrosomites described by Graber (8) and (9) for *Stenobothrus* and other forms.

Later embryos exhibit a progressive increase in the length and complexity of the germ-band.

When the hind end of the band has pushed forward along the dorsal surface of the yolk almost to the anterior end of the egg, three additional segments have been added. These are the two posterior thoracic segments and the first abdominal, and they are added successively from before back; since I have embryos in which the first thoracic is the last segment distinguishable, others with an indistinct second thoracic behind this, and yet a third lot with three distinct thoracic and an indistinct abdominal segment. In older embryos more abdominal segments are added behind. A "tail-piece" of unspecialized (ecto- and mesoderm) material is found at the end of the band during this process, the abdominal segments being successively differentiated from its anterior edge. (*See final section of this paper and final plate.*)

Graber's (9) beautiful figures of the development of the Orthopteran, *Stenobothrus*, would serve fairly well, in most respects, to illustrate the general features of the growth of the germ-band of the Termite from a disc-like rudiment to an elongated, segmented embryo at the period of "revolution." This process was not observed by Graber in *Stenobothrus*. It



should be remembered, though, that the Termite's germ-band exhibits no "macro-somites" of Graber, and that the disc lacks the prominent gastrula groove of *Stenobothrus*.

As a whole, the resemblance between the Orthopteran and the Termite during the embryonic stages is striking.

A stage corresponding to that figured by Brandt (3), Fig. 11, for *Calopteryx* is reached, with the appearance of the mouth and the labrum, and the subsequent folding of the head up from the surface of the yolk. At the same time the segments and appendages have become more prominent.

The embryo, unlike the *Libellulid*, is not immersed in the yolk. (See Pl. XXXII, this paper, also Korschelt and Heider (17), figures on pp. 774, 776, and 777.)

In the Termite, when the germ-band has grown along the dorsal surface of the yolk to the anterior end of the egg, the posterior portion of the abdominal region sinks slightly into the yolk. As the embryo continues to elongate, this bend in the abdominal region becomes more marked, the tail-end of the band coiling ventrally into nearly a complete circle. (See diagrams, Pl. XXXII.)

This caudal flexure is a very characteristic phenomenon. It occurs in many insects and is much like that of the *Libellulid*. (See Korschelt and Heider (17), figures on pp. 774, 776, and 777.) I cannot explain it. It certainly appears to take place here (as in the *Libellulid*), without being necessitated by any combination of mechanical forces that can be stated.

The formation of this flexure has furnished me with a warning, and a good example of what at first sight appears to be a simple mechanical process, but proves to be a phenomenon not so readily dismissed. In many specimens, a very plausible explanation of it seems to be the resistance offered to the posterior end of the elongating germ-band by the chorion, lying at right angles to its course at the anterior end of the egg. This will not serve as an explanation, however, since in many preparations the flexure occurs before the anterior end of the egg is reached (as in the *Libellulids*). It is clear in one instance, at least, that the tail end of the embryo might grow back on the surface of the yolk around the anterior pole, as in

some insects. There was no caudal flexure in this specimen, the hind end of the band turning part way over the pole.

At the completion of the elongation of the embryo the appendages have become quite long. The head is enlarged and globular. The first maxillae are tri-lobed, and the second pair less markedly so. In the abdominal region ten well-marked segments have become established, each with a distinct pair of appendages. No appendages are figured by Brandt on the abdomen at the corresponding stage of *Calopteryx*, and none of the figures of Graber to which I have referred exhibit such well-marked rudiments in that region.

From this stage until "revolution" the embryo undergoes but little change externally, though the sides of the band grow dorsalward, and the appendages elongate considerably.

"Revolution" is accomplished as described and figured by Brandt (3) (also see Korschelt and Heider (17), figures on p. 777) for the *Libellulid*. The amnion and serosa fuse into a single membrane at one point, only to tear open over the ventral side of the embryo and retract dorsally, to finally form the "dorsal-organ" at the back of the head (stages *O* and *P*, Pl. XXXII). The head of the embryo now slips up along the ventral surface of the yolk to the anterior end of the egg, while the tail end comes to lie beneath the micropyles at the opposite end (see diagrams, Pl. XXXII). The ventral surface of the embryo is now entirely on the micropylar, ventral side of the egg, as was the case until after the closure of the amniotic cavity. The embryo has, therefore, returned to the orientation of its first rudiment, the germ-disc.

The remaining processes, up to hatching, consist in the closure of the body along the dorsal mid-line, the completion of the appendages, and the continued development of the internal organs. In the stages following "revolution," the embryo increases so greatly in bulk that, just before it leaves the egg, this has become distended to a remarkable size as compared with the unsegmented egg.



GENERAL CONCLUSIONS AS TO THIS TYPE OF INSECT  
DEVELOPMENT.

Korschelt and Heider's recent text-book (17) contains an argument for a modification of Will's and Wheeler's well-known theory of a connection between the "invaginate," "immersed" type of development exhibited by the Libellulids and some Hemiptera, and the type followed by myriopods.

On p. 775 (17) we find: "The invaginate type is best seen in the Libellulids, which represent the direct connecting link (Anschluss) with the phenomena exhibited by the myriopods, and hence must be regarded as the more primitive type." Again on p. 787 (17): "Wir haben oben gesehen dass bei den Myriopoden bei fortschreitenden Längenwachsthum des Keimstreifs derselbe in seiner Mitte eingeknickt und in das Innere des Eies versenkt wird. In dieser Einsenkung, welche wir uns zunächst durch das räumliche Missverhältniss zwischen dem langgestreckten Keimstreif und der rundlichen Eiform entstanden zu denken haben, werden wir (wie dies schon Graber andeutete und Will neuerdings ausführlicher begründet hat) den Ausgangspunkt für die Entwicklung des invaginirten Keimstreifs der Libelluliden zu suchen haben. Wir werden demnach für die Entwicklung des Insecten Keimstreifs die Form der Invagination als die ursprüngliche betrachten." This account is apparently based on Heider's (13) discussion of the subject in his monograph on *Hydrophilus*. It is a modification of Will's (27), also Wheeler's (25) theory, against which in its original form Graber (9), in a more recent paper than the one referred to before, brought forward strong objections.

Since the publication of the text-book of these two authorities on insect embryology, further investigation has shown, that besides Oecanthus which is mentioned in it, a number of Orthoptera, as well as the Termite (which is strikingly orthopteran), exhibit developmental phenomena similar to those of the Libellulids. It now seems evident that there are no grounds whatever for regarding the method of development followed by this latter group as at all more primitive than that observed for

Oecanthus, Gryllus, or the Termite. These forms should be looked to for a connecting link, if one exists (on this question refer to the discussion of the origin of the amnion in insects in the last division of this paper), between the phenomenon of "doubling-up," exhibited by the myriopod embryo, and the formation of an amnion in the Pterygota.

There is much reason for believing the development of the Libellulid to be secondary, since the embryo is of the "immersed" type.

A. A superficial germ-band is generally characteristic of Arthropods, and when we find one sunken into the yolk, there is cause to believe this position has been assumed secondarily. Among the insects, most forms (and especially the Orthoptera and Termites) agree in having superficial embryos. The exceptions are rather marked, and are found among the Lepidoptera, Hemiptera, and Libellulids. In the Lepidoptera, as in the Myriopoda, the "immersed" position is admitted to have been secondarily derived from the superficial for protection, nutrition, or some other unknown cause. It appears to me most probable that the same is true for the Libellulids and the Hemiptera, with inner germ-bands.

*Hence I should regard the superficial embryos of the Orthoptera and the Termite as more typically primitive for insects.*

B. A striking character of the development of the Termite is the small size of the first rudiment of the embryo, the germ-disc, when compared with the definitive length of the embryonic band. The primary rudiment must elongate through the whole length of the egg, and add successively all the segments of the body before the embryo is fully formed. This is equally noticeable in the case of some of the Orthoptera, but is less pronounced in most insects, particularly among the more specialized forms of the group. In these there is a tendency toward a formation of the embryonic band in its full extent from the start.

*Now it seems to me that the Termite and the Orthoptera, with a superficial embryo beginning in a disc which must elongate considerably to attain the definitive number of segments, have adhered most nearly to the typical method of development for Arthropods, and probably best represent the embryonic develop-*

*ment of the ancestral insects.* The facts of the development of the Crustacea, Palaeostraca, the Arachnids, and the Myriopods (see Korschelt and Heider (17), p. 741) show a similar disproportion in size between the primary rudiment and the definitive segmented adult. This may be illustrated from the Arthropods by referring to the growth of a Nauplius into its adult form. A similar method of growth is found in the development of the Annelid from the Trochophore, where also growth is uncomplicated by the presence of yolk. I do not mean to raise any question of homology between the primary disc-shaped rudiment of the insect embryo, and either the Nauplius or the Trochophore, but to point out that a certain few insects (Termites, etc.), otherwise primitive, have retained a method of growth (see closing paragraphs of this paper) fundamentally similar to that followed by other segmented forms. In most insects, and particularly in the more specialized forms, the formation of a segmented embryo is more direct, a rather long germ-band being established from the first (and, as I take it, precociously), of more nearly the definitive length of the embryo. (*Note that Graber's (9) classification of germ-bands is not here accepted.*)

*C. These primitive forms (Orthoptera and Termite) are also characterized by another peculiarity of interest in the present discussion. The amnion arises very early and completely covers the embryo soon after its appearance as a small disc. We do not know with certainty to what need of the embryo the amnion responds, but we are not surprised to find it in its most primitive condition in the very forms under consideration, which are primitive in so many other morphological characters. I believe this is the case, and that insects, in which the membranes become prominent and cover over the embryo comparatively late in its growth, represent a secondary condition. If, as is generally supposed, the amnion arose as a protection for the germ-band against mechanical injury or too rapid evaporation, or as a sac, to receive accumulated waste products, as Wheeler (25) suggested, it would have been a great advantage for it to appear in the ancestral Pterygota at the earliest possible moment in the growth of the embryo. This moment occurs when the first rudiment of the embryo, the germ-disc, is established and about*

to grow into the elongated segmented embryo. From this time a superficial germ-band would be constantly exposed to the dangers mentioned. Hence the invagination, at this period, of a part of the disc, resulting in the formation of the amnioserosal fold.

The Termite and some of the Orthoptera (*Stenobothrus*, *Gryllus*, etc.) have best retained this method of the formation of the amnion.

In other Orthoptera, the Libellulids, some of the Hemiptera, and many other insects, the ancestral history is not so well preserved. In these the amnion no longer closes over at the earliest possible stage. Wheeler's figures of the germ-bands of *Blatta* and *Doryphora* (25), Graber's of *Lina* (9), Heider's of *Hydrophilus* (13), and Weismann's of *Chironimus* (24) illustrate its usual late closure.

The Libellulids and some of the Hemiptera retain to a decided degree ancestral characters, but the much-retarded closure of the amniotic cavity, and the presence of the so-called secondary "head-fold," together with the marked secondary "immersed" position of the germ-band, render these forms less typical examples of the probable primitive method of development. (*Refer to the discussion of the origin of the amnion in insects, in the last division of this paper, for further consideration of these questions.*)

#### THE ORIGIN OF THE MESODERM IN INSECTS.

Recently the origin of the under-layer in what are regarded as the most primitive insects, the Orthoptera, has been carefully studied by two well-known investigators who have reached quite contradictory results.

Wheeler (26), in his "Contribution to Insect Embryology," has devoted considerable space to a review of the question. His conclusion is expressed in these words: "It follows from the observations here recorded, fragmentary as they are in many respects, together with Graber's observations on *Stenobothrus*, that the Orthoptera can no longer be regarded as *hors de ligne*, so far as the formation of their germ-layers is concerned. In













all the families of the order, save the Phasmidae, an invaginate gastrula has been found, and there can be little doubt that the investigator who is so fortunate as to study embryos of this family will find in them essentially the same process of germ-layer formation. The view is now pretty generally held that in the Insecta both mesoderm and endoderm arise from a median longitudinal furrow (the former layer throughout nearly the entire length, the latter only in the oral and anal regions of the germ-band), and that vitellophags, or cells left in the yolk at a time when the remaining cleavage products are traveling to the surface to form the blastoderm, take no part whatsoever in the formation of the mesenteron, but degenerate *in situ* and finally undergo dissolution."

I have been unable to obtain a copy of Heymons's study of the germ-layer formation of Orthoptera and Dermaptera (14), but his conclusions have appeared in abstracts and are as follows: The yolk-cells take no part in the formation of the embryo. There is no true gastrulation process, but the under-layer arises from all parts of the embryonic area. When what is usually regarded as a typical gastrula invagination occurs, as in most insects, it is to be explained, not as gastrulation, but as a simple mechanical process caused by an aggregation of cells at one point. The layer generally known as the mesenteroderm is in reality only mesoderm, the endoderm appearing relatively late and arising from the ectoderm of the stomodeal and proctodeal invaginations.

*My results agree with Heymons's conclusions as to the origin of the mesoderm of insects primitively in a collection of cells arising diffusely from the ectoderm; but I must differ from him and agree with Wheeler in the latter's interpretation of the invaginate groove, from which the endoderm and mesoderm arise in most insects, as a true gastrula.*

The Termite, which is certainly as primitive as any other insect hitherto described, exhibits no gastrula invagination. I have shown that the under-layer begins to appear at all points in the embryonic rudiment at an early stage of its formation. The plug of lower-layer cells, which becomes so prominent as the germ-disc grows more distinct, is apparently largely

the outcome of concentration of the cells of the disc toward the center. The relation of such a manner of formation of the under-layer to that generally described for insects is interesting to consider. This process does not appear to me to be derived from an invagination as a slurred gastrula. It is rather a method of delamination, where there is a further tendency in the lower-layer cells to collect toward a middle point.

A similar method has been described for Crustacea, Arachnids, and Myriopods, and all of these facts, taken together, lend weight to Heymons's contention that an indefinite migration below is the more primitive method of forming the under-layer in insects.

Heymons's explanation of the gastrula groove commonly found in insects, however, requires examination.

He does not attribute to such invaginations the significance of a process of gastrulation. From his standpoint the invaginate groove (which, as Wheeler points out, is so universally present among insects, and so essentially involved in the establishment of the under-layer) is a mechanical process and independent of the formation of mesoderm or endoderm.

I do not see the strength of this position.

In so far as this author finds the diffuse method of the origin of the mesoderm in certain Orthoptera the primary one, and offering a favorable basis for the origin of an invaginate gastrula, he seems justified.

I cannot, however, take the further step with him and dismiss the invaginate gastrula, found so universally among insects, as no gastrulation, but as simply a result of the crowding of an aggregation of cells at one point. Though we still have such an aggregation in the Termite, it has not in this group led to invagination as a mechanical necessity. In the place of invagination there is simply a crowding of certain ectoderm cells, arising at irregular points, below into a solid plug extending down into the yolk.

As far as our understanding of mechanical forces and their necessary results goes, the reason is not clear, without further addition, why the mesoderm came in other forms to arise in a groove instead of continuing to wander below in a solid mass.

That the under-layer is formed most easily and efficiently by a process of invagination seems evident, from the almost universal appearance of the gastrula groove in insects. Given first the more primitive, diffuse method of forming this layer still persisting in the Termite and, as Heymons claims, in other primitive insects, we may attribute to Natural Selection its improvement until an invaginated gastrula groove has become the common and readiest means of attaining the end. When we use Natural Selection as the agent of this change, we of course mean that the primary organic structure (in this case the mesodermal cell rudiment arising diffusely from all points of the ectoderm) was forced to respond to a further combination of forces in the environment which we cannot define in more exact physical terms.

From this point of view the usual method of forming the mesoderm in insects, by a well-marked gastrula groove, is not an independent or accidental phenomenon, but has been derived from a more primitive method of migration already established in the earlier insects, not as a direct and necessary result of apparent and readily stated mechanical conditions, but as a response to additional forces, compelling an important change in the older but less direct process which is still efficient in some primitive insects. These "additional forces" (mechanical, chemical, or what not), included under the general term "adaptive," did not "necessarily" disturb in the Termite the primitive habit established in their ancestors. In other insects, when new conditions (mechanical or others) made it possible and more desirable, invagination arose as a response.

A study of the origin of the "lower-layer" in the Termite shows a very close connection between this and the establishment of the first rudiment of the embryo by a concentration of the blastoderm cells toward a certain area (as in the case of Isopods discovered by McMurrich). This more general phenomenon must be first explained before attempting the special problem of the exact mechanical nature of the origin of the mesoderm, which is too intimately bound up with the solution of the former question to be considered alone.

As to the entoderm of the Termite, I must say that it

appears late, after the segmentation of the germ-band. The yolk-cells (as both Heymons and Wheeler claim) can take no part in the formation of this layer; since at an early stage, before the closure of the amniotic cavity, they have become very large and unlike the cells which later form the entoderm.

The fact that this layer arises so constantly among insects with the mesoderm at the two ends of the invagination, termed "gastrula" (see Wheeler (26)), is a strong point against Heymons's assumption of the independent, accidental character of this groove.

I shall be obliged to defer to another time the discussion of the method of the origin of the entoderm, its exact relation to the mesoderm and to the gastrula groove, when this occurs, as well as its association with the stomodeal and proctodeal invaginations.

#### THE ORIGIN OF THE AMNION IN INSECTS.

The discussion as to the cause of, and the primitive method of origin of, the embryonic membranes of insects has at least developed some extremely interesting ideas.

At present, opinions seem to halt between, first, the Ryder-Wheeler (26) hypothesis of a purely mechanical and independent origin of the amnio-serosal fold among the winged insects; and, second, the theory of Will (27), Wheeler (25), and Korschelt and Heider (17), recently championed by Heymons (15), which associates the formation of embryonic membranes in insects, more or less closely, with a certain phenomenon exhibited by the myriopod embryo. Wagner's (23) views I shall put, for convenience, in the first category; while Willey's (29) recent contribution, though in some respects agreeing with the second, will have to be considered alone.

A. Examining first the Ryder-Wheeler theory, we find that Wheeler (26) has adapted Ryder's (22) "mechanical explanation" for the origin of the amnion of vertebrates to the insect amnion. Of course the term "mechanical" is here used in its narrower sense, referring the question to immediate antecedent causes, which alone are claimed to necessitate the result. The



question whether the origin of organic structures is ultimately purely a problem of mechanics, as a first cause, is not raised. Here the contention is that certain evident and simply stated conditions of pressure and mechanical strain are alone sufficient to force the amnio-serosal fold to arise.

Wheeler (26) advocates this idea concisely, as follows: "The amnio-serosal fold is a mechanical result of a local induplication of the blastoderm, due to rapid proliferation in a single layer of cells." "There is the vesicular one-layered blastoderm filled with yolk, and the germ-band arising by rapid proliferation at one point. The resistance of the yolk being less than the external resistance of the tightly fitting chorion and vitelline membrane on the one hand, combined with the peripheral resistance of the extra-embryonal blastoderm on the other, the germ band is forced to invaginate. This invagination is favored by the displacement of yolk during its liquefaction and absorption by the growing embryo. We may suppose that this invagination, which results in the formation of the amnio-serosal fold, assumed a definite and specific character in different groups of insects."

Similar mechanical conditions are appealed to as the cause of certain invaginations in other forms; the Cestode head in *Cysticercus*; the Nemertine in the *Pilidium*; the formation of the young *Spatangid* in the *Pluteus*; the development of the amnion and serosa in vertebrates; and the imaginal discs of insects.

a. 1. Even if we admit the presence of just such a combination of forces as is enumerated above, they seem to be subsidiary and insufficient alone, without a further cause, to explain the origin of the membranes for the following general reasons:

It must be recalled that no amnion results in the similar rapidly proliferating areas of crustacean eggs, that such a membrane is lacking among the myriopods and apterygote insects (in spite of Heymons's (15) claim, which requires further and more convincing proof, as we shall see later), and that it is not formed in certain of the higher insects. It should also be remembered that similar membranes are want-

ing in anamniote vertebrates, where the mechanical conditions, as far as this theory goes, appear to be much the same as in amniote vertebrates.

Apparently similar conditions of pressure and mechanical strains would be brought to bear on the embryonic areas of the myriopods, the apterygota, or crustacea, as are claimed to necessarily force the formation of the amnion of insects, but no amnion appears in the former groups. The invaginations which do occur (to form the eyes, the digestive tract, etc.) in some of the rapidly proliferating areas of the decapod blastoderm would be generally thought to necessitate something more than such an enumeration of mechanical strains to explain them.

In those highly specialized insects that entirely lack an amnion, its failure to appear is even more marked. Here, within the same group, there are forms which, in the face of the forces above stated as sufficient to produce an amnion, have none.

The effort to apply such a simple mechanical explanation to the origin of various organic larval structures may seem plausible at first sight; but, carried to its logical limit, not so much so. Why stop at the structures mentioned? Might not the germ-layers, the central nervous-system, as well as other such rapidly proliferating areas, be as readily included? Heymons, as I have shown, has already attempted an affirmative answer for the origin of the gastrula groove.

*a.* 2. Turning from such general considerations to my own special results, the formation of the caudal flexure of the Termite seems a case in point.

This ventral flexure of the tail end of the embryo, as I have pointed out, at first seems just as reasonably to be ascribed, solely and directly, to a necessary result of pressure or mechanical strain as the instances referred to by Wheeler. A single unusual specimen proved beyond doubt such a conclusion to be false, and that what might appear superficially to be a necessary method of growth could be accomplished in an entirely different manner. It was certainly proved to be independent of the resistance of the chorion, which seemed so determinative at

first sight. Here was another case of the nearest explanation not necessarily being the true one.

It seems hardly necessary to say that the fact that such invaginations can be watched step by step sometimes, and can be actually observed to encounter resistance at every stage, is no proof that such resistance causes the process.

a. 3. My study of the formation of the embryonic rudiment and of the origin of the amniotic fold of the Termite indicates forces of a very different nature from those formulated by Wheeler; in fact the very reverse.

As the germ-disc becomes sharply defined, the area of the blastoderm occupied by it is distinguished by the closer crowding of its cells, while the surrounding cells become flattened and pulled apart into a thin membrane. There appears to be a contraction toward the embryonic area, as is observed in the formation of the embryonic rudiment in other insects and other arthropods. At any rate, the extra-embryonal blastoderm may be said to be stretched and kept so by the changes taking place in the embryonic area.

Before the amnion arises it is clearly differentiated as a special thickened area of the germ-disc. When the embryonic rudiment doubles-up, and this posterior portion of it folds over to become the amnion, the extra-embryonal blastoderm is pulled forward and further stretched.

*It seems correct to speak of the tension of the serosa as due to the activities in the embryonic area, rather than to reverse the case and explain important changes in this area as a result of such tension.*

In studying the growth of the germ-disc, I can find no indications of a rigid resistance to its growing edges claimed to be offered by the rest of the blastoderm. The cells around the rapidly proliferating area do not seem to be fixed, immovable points; and the membrane they form does not appear to be more resistant to this more active area than is the yolk.

Another important point is the fact, as I have shown, that the amnion is not a derivative of the extra-embryonal blastoderm, as Wheeler (26) concludes in his latest paper.

If the Ryder-Wheeler mechanical theory were correct, the most natural place to expect the fold would be just at the junction of the rapidly proliferating germ-disc with what is claimed as a rigid, resistant, extra-embryonal region. We would look for the weakest point here. The fold does not, however, occur here in the Termite or other amniote insects.

My own observations, and a general review of the question, lead me to believe that the embryonic membranes of insects are adaptive structures, which arose in the winged insects as a response to some definite need of the embryo. I do not think the exact combination of physico-chemical forces, coöperating to bring about this result, can be stated at present. The eggs of the anamniote apterygota are, to all appearances, as far as mechanical conditions go, similar to those of winged insects. The physical constitution of the egg was already favorable to the origin of the amnion in the ancestors of the latter forms; but before one arose, certain additional forces were necessary, which must be associated with some necessity of covering over the embryo at an early stage. Whether this necessity (physico-chemical, no doubt) was one of protection, prevention of evaporation, better nutrition, or to furnish a depository for waste products, may not be decided; but any one of these suggestions, or all together, would be reasonable cause.

When forms arose among the higher insects, as adaptations to special new conditions, the early completion of the process became less important; and in a few cases the amnion ceased to appear, being no longer needed. (If it is any more precise, we may say that the amnion was no longer maintained by the physico-chemical forces which originated it.)

*B.* I must refer to Wagner's (23) comprehensive theory of the origin of insect embryonic membranes and other organic structures, as another example of a simple, clear-cut mechanical explanation of such problems, which also illustrates the difficulty of correctly estimating and balancing forces, and their necessary effect on organized matter.

In a few words, his idea is as follows: Think of the similar cells of a uniform epithelium as an organic molecule, so built











up together that a certain homogeneous reciprocal relation is attained. Now, when certain cells of this layer become altered in nature under the influence of some special forces, the reciprocation with neighboring cells is likewise altered, resulting in so changing the relations with these latter that a separation of the changed cells from the layer of similar, unchanged cells must take place.

The mechanical basis of the theory is what happens when a foreign, inorganic particle is introduced into a fluid or viscid layer ("Haut") whose elemental drops coöperate reciprocally to form a uniform sheet. The foreign particle would be thrown out as a result of purely mechanical tensions.

So in the case of the origin of the germ-layers, by invagination or immigration; the sub-epithelial muscle cells of Medusae; gland cells; central nervous-system; sense organs; Cestode head in the cysticercus; the extra-embryonal and embryonic cells; embryonic membranes; imaginal discs, etc. Whenever two kinds of cells occur in an epithelial layer, one sort is thrown out, so to speak, by invagination or immigration. Cases where this has not taken place represent the early stages of the process (as certain epithelial gland cells or muscle cells). In all these cases the common and necessary cause of invagination and immigration is claimed to be the sharp differentiation of certain cells, physically and chemically, to such an extent that they must move from their primitive position.

It seems hardly necessary to observe that, though this theory is strictly logical and far-reaching, Wagner does not explain the fundamental question why certain cells rather than all are modified; and that he overlooks an important and essential difference between the living modified cell in the uniform cell layer, on the one hand, and the foreign, inorganic, dead particle in the homogeneous fluid layer on the other. The theory must collapse when we reflect that, instead of being obliged by the supposed necessity to immigrate as an inorganic particle, the modified living cell could accommodate itself to its old neighbors and remain with them. Of course the inorganic particle would have no such power of adaptation resident in living protoplasm. Possibly this adaptability of living substance

is the reason Wagner finds gland cells, muscle cells, etc., not wandering out of the otherwise uniform layer. When a migration of specialized cells does take place, we shall have to look further than to such a simple statement of inorganic physics for the explanation.

C. I shall not examine Willey's (29) hypothesis at length, since, in as far as it refers to the origin of the amnion, it appears to be largely a statement of Heymons's (15) views, which will be considered further over.

Willey's main thesis seeks to prove, by reversing a theory of Hubrechts's, that the extra-embryonal blastoderm of the insect egg (*i.e.*, the serosa and amnion) is a secondary cellular membrane, derived in a curious roundabout manner from a more primitive, extra-embryonal trophic membrane, "the trophoblast"; which, "as it is preserved to us in the embryo of *Peripatus novae-britanniae*, arose in adaptation to a viviparous habit acquired by the terrestrial descendant of an aquatic ancestor; and that it became transformed, whether directly or by substitution, into the serosa, in correlation with the secondary deposition of yolk-laden eggs."

The following fundamental assumptions seem to me inadmissible: That the viviparity of *Peripatus* is primitive; that "lecithality and deposition of the eggs of insects are both secondary"; that this application of the idea of substitution involving the reverse of Hubrechts's idea is reasonable; that the amnion is a derivative of the extra-embryonal blastoderm in insects; or that the serosa of the insect egg has any such indirect phylogenetic history, believing it as I do to be directly comparable to the inactive extra-embryonal surface cells (Deck-schicht) of other yolk-laden eggs.

D. 1. I have already discussed, in the division of this paper headed General Conclusions as to this Type of Insect Development (page 29), certain aspects of the theory presented in Korschelt and Heider's text-book. Reference must be again made to that section of my paper, where the original sources and criticisms of the theory are quoted. This theory, which originated with Will and Wheeler (25), was later modified by Heider.

The origin of the embryonic membranes of insects is referred to the peculiar phenomenon of "doubling-up" exhibited by the myriopod embryonic band.

It was originally claimed by Will that the invaginations in the two cases are genetically connected to such an extent that some of the posterior segments of the elongated myriopod band, on bending forward, were directly transformed into the amnion in the ancestral insect embryo. In this way the adult insect came to have fewer posterior abdominal segments than the myriopod.

Judging from Heider's remarks in his monograph on *Hydrophilus*, no genetic relationship is now meant to be implied between the "doubling-up" of the myriopod embryo, and the invagination to form an amnion in the insects.

The idea now advanced is, that the resemblance in the two cases is of sufficient importance to intimately connect the phenomenon presented by the elongating myriopod embryo with that observed in Libellulids, which are said to best exhibit what is termed the primitive, invaginate type. The similarity in the two instances, however, is only claimed to be the result of the action of a common cause of the invagination.

Will's idea of a transformation of a part of the segmented body of the embryo of the myriopod into an amnion for the insect embryo has been abandoned since Graber's (9) criticism.

Heider in his monograph (13), and in the text-book with Korschelt (17), points out the similarity between the amnion and the rudimentary ectoderm of the embryonic band from which it arises; and in this is in agreement with what has been observed and figured by most investigators, as I have already noted in another connection.

D. 2. Recently, Heymons (15) has studied the interesting apterygote *Lepisma saccharina*. As a result, he claims to have furnished us with a convincing intermediate stage between the phenomenon of doubling-up of the myriopod embryo, and the formation of an amnion and amniotic cavity in winged insects; which he thinks proves that the latter process is directly derived from the former.

In describing the embryonic rudiment the author speaks



of the entire extra-embryonal region as a serosa, before the doubling-up takes place. As this happens, he says (in a paragraph on page 587 of his paper) that the cells of the edges of the embryonic band become pulled out into a thin cellular membrane, the amnion. None of his figures, however, give proof of such a process of transformation of a part of the

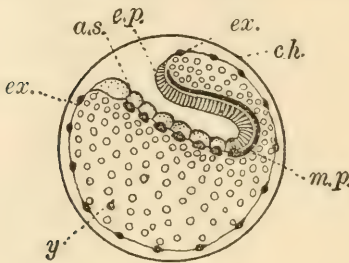


FIG. 1.

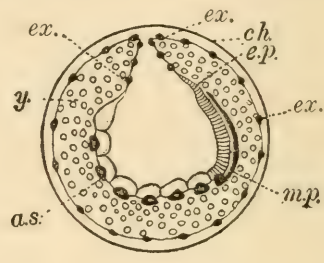


FIG. 2.

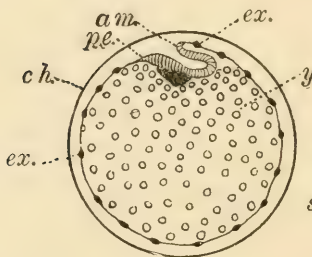


FIG. 3.

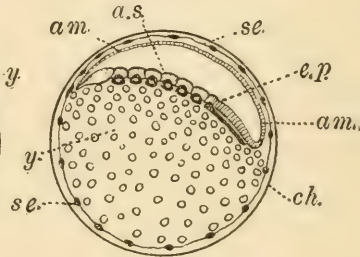


FIG. 4.

Diagrammatic figures (sagittal sections), comparing the "primary ventral flexure" ("doubling-up," or invagination) of the myriopod and apterygote embryos with the amniotic fold of the winged insect. (1) Myriopod (*Julus*) embryo, at the first appearance of the ventral flexure; (2) similar stage of the embryo of *Lepisma* (apterygote); (3) early amniotic fold (doubling-up, or invagination) of the unspecialized embryonic rudiment of the Termite; (4) later stage of the Termite embryo, after the closure of the amniotic cavity—a stage similar to that of the two types in 1 and 2, but the "tail-piece" is straight here. *ex.* extra-embryonal blastoderm; *e.p.* posterior, unspecialized ectoderm; *m.p.* posterior, unspecialized mesoderm; *a.s.* segmented region anterior to unspecialized tissues of "tail-piece"; *ch.* chorion; *y.* yolk; in 3, *p.e.* primitive, unspecialized ectoderm of germ-disc; *am.* amniotic fold in undifferentiated ectoderm; in 4, *se.* serosa; *am.* amnion.

embryonic, rudimentary ectoderm into the amnion, as observed in the winged insects. The figures show no more as to this question than that, as the embryonic band sank into the yolk, the extra-embryonal cellular membrane attached to its edges was pulled along.

The result, as figured, is a band doubled-up like that of the myriopods, and not differing from this except in lying deeper in



the yolk, into which some of the extra-embryonal membrane is dragged down. (See my Text-Figure 2.)

Comparing the diagrammatic text-figures here copied, — one, Fig. 1, after Heathcote (31), Fig. 14, showing the doubling-up of the myriopod (*Julus*) embryo, and another, Fig. 2, after Heymons (15), for *Lepisma*, — we find the thick germ-band in each case enclosing a cavity. In the myriopod egg the two ends of the embryo are on the surface, and there pass into the thin extra-embryonal membrane. In the *Lepisma* egg the embryo lies more internally. Except for this, there is no essential difference apparent in the relations of the extra-embryonal blastoderm to the embryo or in the nature of the open cavity. Compare these two figures with the third text-figure of the Termite, at a stage before the closure of the amniotic cavity, and with the fourth figure of the closed amniotic cavity of the Termite. (All eggs are represented as spherical for better comparison.)

Does the so-called amniotic cavity of *Lepisma* constitute any nearer approach to the true amniotic cavity than the one previously found between the “doubled-up” body of the myriopod embryo? I think not, without a further and more convincing series of figures of some stages in the formation of the so-called amnion, which would prove it to be any more truly an amnion than the part of the blastoderm external to the embryonic band of the myriopod, or in any way different from this, except in being pulled down into the yolk.

Instead of being an important intermediate stage between the phenomenon exhibited by the myriopod embryo and the formation of a true amnion, and amniotic cavity, in the winged insects, there is nothing in the figures (nor does the single descriptive paragraph convince without further figures) to give reasonable grounds for the claim that there is any such difference between the phenomenon exhibited by the myriopod and that shown by Heymons for *Lepisma*.

The gap between the open cavity in the doubled-up myriopod embryo and the true, closed amniotic cavity of winged insects, seems just as wide as before *Lepisma* was studied from this standpoint; except in as far as the apterygota have been

shown to exhibit this phenomenon similar to the myriopods — an important point in itself, indeed, if the amniotic fold of the winged insect is to be derived from an earlier invagination.

Willey differs with Heymons as to interpreting the ventral flexure of the embryo of *Lepisma* as comparable with the later caudal flexure of insect embryos. Without admitting his theory of the trophoblast, I must agree with Willey in this distinction.

In many respects the development of *Lepisma* bears a close resemblance to that of the primitive Orthoptera and the Termite. It is interesting to find the germ-disc originating at the posterior pole of the egg as in the Termite. The absence of a gastrula groove, in connection with the origin of the mesoderm, is also in agreement with what Heymons has found in some Orthoptera, and with the results here submitted for the Termite.

*E.* The conclusions reached from the above general review of the question before us, in the light of my own special observations, and again referring to my views, expressed in a previous section of this paper, as to the primitive type of insect development, may be summed up as follows :

*c.* 1. The amniotic fold did not arise as a necessary result of any combination of purely mechanical forces which has been formulated up to the present time.

*c.* 2. The amnion and amniotic cavity of insects are adaptive structures, which, as far as our knowledge now goes, arose first in the winged insects as a response to some definite need of the developing embryo.

*c.* 3. The amnion is primitively a derivative of the rudimentary embryonic ectoderm.

*c.* 4. An "invaginate" type of development is the more primitive one for insects. Irrespective of its relation to the phenomenon of doubling-up of the myriopod or apterygote embryo, it has been shown to be associated with the more primitive insects, and the most primitive (probably) method of membrane formation outlined in paragraph 5 below.

*It must be added to this, that in the light of researches of a more recent date than that of the publication of the text-book of*

*Korschelt and Heider, it is found that the Termite and certain Orthoptera with superficial embryos, as is explained in this paper, represent the invaginate type of development there suggested better than do the Libellulids, with embryos "immersed" in the yolk and other secondary characters. Other methods of origin of the amniotic fold are most probably derived from that best exhibited by the Termite and certain Orthoptera.*

*e. 5. It became important for some reason (whether for protection, better nutrition, accumulation of waste products, etc.) associated with a new habitat or mode of life, that the superficial embryos of the ancestors of the winged insects should be completely covered over. The forms we may now consider primitive for a number of reasons exhibit a relatively small, superficial disc as the first rudiment of the embryo. Here was an especially favorable condition for the earliest possible appearance of the membranes, at a time when they might be particularly needed. Only a few forms have retained this process in a near approach to its primitive form. (I believe that the amnion is formed from the rudimentary ectoderm by essentially the same method, on the similar germ-discs of the Termite and certain Orthoptera, though in the Termite the fold is more evident at the posterior end.) Changed conditions have led to a disappearance of the membranes in a few insects.*

*e. 6. The ventral flexure of the first rudiment of the embryos of the invaginate type which forms the amniotic fold has not been proved to be of a phylogenetic significance.*

*e. 7. Even if it can be shown conclusively, in the case of the apterygote egg, that the open cavity is a somewhat nearer approach to the amniotic cavity of the winged insect than that found in the myriopod egg (or, in other words, what Heymons speaks of as amnion is a derivative of the rudimentary ectoderm, as in the Termite, and not simply a part of the blastoderm comparable to that lying outside the limits of the embryo in the myriopod egg), it must be remembered that the open invaginations of the myriopod and apterygota may not even be due to causes similar to those calling for the closed amniotic cavity of winged insects. These may be entirely distinct phenomena with very different significance.*

There is, however, undoubtedly a resemblance between this invagination and the phenomenon exhibited by the myriopod embryo, which is strengthened by the appearance of the same condition in the apterygote egg. This suggests strongly a common cause (the general adaptive nature of which I have suggested in agreement with others already quoted); but Korschelt and Heider's ((17), pp. 734 and 787) further idea, that this cause is associated necessarily with the resistance offered by the spherical chorion to the growth of the elongating germ-band, does not seem convincing, since these authors themselves suggest an objection in the different behavior of some myriopods, see (17), p. 735; since similar conditions do not necessitate a like invagination in certain insects, or in the elongating band of the arachnid or in that of a fish, for reasons I have suggested in another place; and since the amniotic fold of the Termite arises on the nearly circular disc, before such conditions would be effective.

*c.* 8. The possibility of a connection with the invagination ("doubling-up") of the myriopod embryo seems sufficiently strong to warrant a new statement of how a fundamentally similar invagination, in the primary embryonic rudiment of the myriopod-like ancestors of winged insects, may have formed a starting point for the formation of an amnion.

As has been pointed out, some theory associating the two invaginations has seemed probable to a number of investigators.

Will (27), basing his theory on a study of the Hemipteran embryo, first insisted on a derivation of the amnion from a region of the myriopod body.

Wheeler (25), at about the same time and independently of Will, advocated much the same idea, though he simply quotes Will in regard to the degeneration of segments into an amnion.

Graber (9) justly criticised the idea of a disappearance of certain posterior abdominal segments of the myriopod-like ancestors of the insects, by a degeneration into an amnion and a forward migration of the anus.

Finally Heider (13), and later Korschelt and Heider (17), presented a modification of the theory I have outlined, which,













accepting a fundamental common cause connecting the two invaginations, abandoned further comparison.

Heymons (15) has quite recently claimed to have carried this a step further, in a manner which I have already considered.

If we proceed from the assumption that some like necessity of removing the embryo from surface insults, or of furnishing it with better conditions of nutrition, etc., caused an invagination of the embryo of the myriopod (or apterygote) and of the superficial rudiment of the ancestral winged insect, it is possible in the case of the Termite embryos at an early stage, just before the closure of the amniotic cavity, to make a comparison of a somewhat different nature from what has hitherto been suggested.

The condition found in the Termite permits us to see how we may retain an essential feature of Will's idea (also Wheeler's) of a derivation of the amnion from a portion of the ancestral myriopod's embryonic tissue, in association with a process of invagination, without involving the further idea of a transformation of definitively organized tissue, with the disappearance of segments and the migration of the anus. It will, however, be found that the following is not an effort to trace the amnion in a phylogenetic sense back to the myriopod.

Referring back to the text-figures, we find practically the same condition in the three first diagrams—a doubled-up, comparatively thick embryonic band, enclosing a cavity which opens on the surface of the egg. In the Termite this opening in Text-fig. 3 closes, and the outer wall of the cavity, which is a portion of the ectoderm of the first rudiment of the embryo, becomes the amnion. (See fourth text-figure.)

It is evident that if the invagination of the winged-insect embryo is to be derived from that in the myriopod (or apterygote) egg, the amnion of the insect most probably arose from some portion of the thickened, unspecialized (striped in the text-figures) ectoderm of the myriopod (or apterygote) ancestor.

My idea is that, since it has been shown that the amniotic fold of the Termite is a specialized portion of particularly the posterior ectoderm of the embryonic rudiment, at a very early stage, before elongation begins and before the appearance of

segments or the anus, the comparison with the embryonic, invaginating rudiment of the apterygota and myriopod should be made with the ectoderm alone, and at a correspondingly early stage in its differentiation.

*My effort is not to derive the amnion from a portion of the myriopod body in a phylogenetic sense; but to explain how, in association with a fold similar to that of the myriopod-like ancestor, but appearing sooner, it may represent an early specialization of the undifferentiated tissue (ectoderm) of a primary embryonic rudiment common to the two arthropods (see B, pp. 30-31); and how this folding off of the amnion need not prevent the usual continuation of the development into an elongated embryo comparable to the myriopod.*

Such a comparison may be readily made by referring back to the diagrammatic text-figures.

These figures of course represent actual stages in the development of the three forms. The two upper figures illustrate the first appearance of the ventral flexure (doubling-up) of a myriopod, Text-fig. 1 (Julus, after Heathcote), and of a wingless insect (the apterygote *Lepisma*, after Heymons), Text-fig. 2.

Following Heathcote (31) in his description of the myriopod development, we find in the first text-figure that the ventral flexure occurs here comparatively late in the ontogeny, after a few anterior segments have been formed from both layers. The important fact to note is that the bending takes place just behind the last segment differentiated, and in a region that Heathcote speaks of as unspecialized tissue, commonly termed the "tail-piece." I have indicated in the diagrams the usual sharp distinction between the early ectoderm and mesoderm in this posterior region, Text-fig. 1 (see Heathcote's Fig. 30). (Note that the as yet undifferentiated ectoderm is striped in the diagram, while the similar mesoderm is a simple black line.)

Examining Heymons's results for *Lepisma*, as represented in the second text-figure (Text-fig. 2) to the right, we find essentially the same conditions as in the myriopod. (See his Fig. 1, (15), for the sharp separation of primary ectoderm from mesoderm.)

Turning finally to the two lower text-figures of the developing Termite embryo, we recall that the germ-disc, when the amnion first folds up and before the closure of the amniotic cavity, is in a very undifferentiated state. (The suggestion is made in a previous section of this paper (*B*, p. 30) as to the comparatively primitive nature of this small rudiment.) I have indicated in the diagram illustrating this stage (Text-fig. 3) that the entire rudiment, ectoderm and mesoderm, is quite unspecialized. The upper layer is striped, as is the ectodermic tissue of a similar early stage in differentiation in the "tail-piece" of the other figures. The lower layer is also an unspecialized mass.

The condition of the tissues is just what was found in a much later stage of the myriopod (or apterygote), in the particular region where the ventral flexure occurs (Text-figs. 1 and 2).

A first difference is, that though the flexure takes place at a corresponding stage in the differentiation of the tissues, it occurs at a much earlier period in the development of the winged insect; in fact, at what I have pointed out is the earliest possible stage for the origin of an amnion. Another and second point is that only the ectoderm is here concerned in the flexure. Thirdly, if a posterior portion of the primary unspecialized ectoderm becomes amnion, what will be the effect on the further development of the embryo?

The first point of difference, the relatively very early appearance of the flexure in case of the insect, may be unimportant; since the two invaginations before us develop at a like stage in the differentiation of corresponding tissue (see text-figures). We have suggested apparently good reasons for an especially early folding, or invagination, of the superficial rudiment of the ancestral winged insect. Text-fig. 3 shows this taking place before any segments have been differentiated.

As to the second point, in regard to the ectoderm, it must be first recalled that Heathcote's Fig. 30 for *Julus* indicates a special participation of the ectoderm in the flexure, when first beginning. Further, in both the myriopod and the apterygote on the one hand, and the winged insect on the other, there is a marked separate though associated development in the upper

(ectodermic) and lower (mesodermic) layers when once established. Each layer develops certain structures peculiar to it (text-figures). The primitive ectoderm alone would, on *a priori* grounds, be expected to be the layer to differentiate a protective structure, as the amnion has been thought to be. Finally, in the Termite the doubling-up to form the ectodermic amniotic fold takes place distinctly before the mesoderm has spread beneath the posterior region, where the process is inaugurated (Text-fig. 3).

The third point suggested was the effect on further development of the early formation of an amnion from the unspecialized posterior ectoderm. It is interesting to observe, as the fourth diagram (as well as the final plate in this paper) shows, that after the formation of the amnion as one of its organs, the ectoderm, as well as the mesoderm beneath, continues to grow posteriorly, carrying the amnion behind and budding anteriorly the ectodermic portions of the segments of the body until, finally, we reach a stage identical with that of the myriopod or apterygote. The unspecialized tail-piece of this stage was formed in the usual manner from the original, undifferentiated, posterior tissue of the primary rudiment, from which the amnion arose at an earlier stage.

In a sense the formation of the ectoderm of the tail-piece, in this later stage of the winged-insect embryo, may be thought of as a regeneration of the lost terminal material which went into the amnion; just as a piece of the ectoderm of a developing hydra (worm, or other form) might be removed at an early stage, without disturbing the further development of parts from the ectoderm, since the ectoderm remaining would supply the loss. This statement must, however, be accepted as an illustration of regeneration from undifferentiated tissue, only in so far as such a process is comparable to normal growth following the differentiation of an organ from unspecialized tissue.

*From this point of view the amnion is not a substitute for, or a transformation of a posterior region of the myriopod body. It is not derived from any previous structure. It is a specialized structure folded off in the winged-insect embryo, for some adaptive reason similar to those causing the doubling-up of the*



*myriopod embryo, at an especially early period, from the primitive unspecialized ectoderm of an ancestral disc-shaped rudiment. It arises especially in a posterior region of the early ectoderm of the Termite. A similar primitive origin may, however, be associated with the sinking of the embryonic rudiments of other insects (especially in the forms I have taken as primitive), where the folding occurs in other portions of the early ectoderm, for it must be remembered that such ectoderm can produce lateral as well as serial organs.*

The less number of segments in the winged-insect or apterygote, as compared with the myriopod, was attained, as far as the embryology shows, by an arrest in a primitive method of growth common to arthropods and similar to budding, which was continued for a longer time in the many segmented ancestors of insects.

The reason for the shorter duration of this process in the later group is not known, but must be sought in such related fundamental problems of growth as regeneration of lost parts, metamerism, and the cleavage of the ovum.

*e. 9.* As has been said, the ventral flexure of myriopod embryos of the present time may be proved later to be connected in no sense with an amniotic fold. Even if such turns out to be the case, the above comparison will then have served a good purpose, in calling attention to a plausible interpretation of the amniotic fold as originating primarily by invagination in winged-insects, independently, and not traceable to any previous similar phenomenon.

*e. 10.* If the above view is applied to the vertebrate amnion, the participation of both primary and, at the point of origin of the fold, undifferentiated layers of the body-wall would be understood in a sense similar to the formation of other early organs, in which both primary layers coöperate.

#### EXPLANATORY NOTE.

This paper was accepted as a thesis, May, 1896. It was abstracted in the *Johns Hopkins University Circulars*, Vol. XV, June, 1896. Unavoidable delay in publishing and a renewed

study of some additional and better material have rendered the present revision advisable. I have hence included a consideration of two recent papers, that of Heymons (15) and that of Willey (29). It is a pleasure to here thank Professor C. O. Whitman for many courtesies extended to me during two summers' work at the Woods Holl Marine Biological Laboratory.

AUGUST 16, 1899.

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## EXPLANATION OF FIGURES.

All figures drawn with aid of camera. From Figs. 1-29 inclusive, about the same magnification. Figs. 1-24 drawn with Zeiss *oc.* 4. *Object. A.* magnifying 97 times. Figs. 24-29 inclusive, drawn with Bausch & Lomb's tube 160 mm., *oc.* 25 mm., *object.* 17 mm., magnifying 96 times. Figs. 30-37 inclusive, drawn to same scale with Bausch & Lomb's *oc.* 25 mm., *object.* 4.2 mm., magnifying 450 times. Tube 160 mm.

## EXPLANATION OF PLATE XXIX.

(The posterior ends of all eggs are placed uppermost.)

FIG. 1. Ventral surface of egg tipped up somewhat to show micropyles at posterior end. *A.*, anterior; *P.*, posterior; 1-9 on each side the micropylar funnels.

FIG. 2. Optical section of egg with one nucleus, segmentation nucleus, in center. Egg stained in borax carmine and viewed as a transparent object in clove oil. Yolk bodies not shown in figure. The chromatin of the nucleus is seen in the center of a small mass of lightly stained protoplasm: *p.b.*, polar bodies.

FIG. 3. Optical section of egg with two nuclei: *p.b.*, polar bodies; *d.n.*, dividing nucleus, in which the chromatin is separated into two masses.

FIG. 4. Optical section of egg with four nuclei. The nuclei are not all in the same plane. A line connecting the two posterior nuclei is in a plane at right angles to one joining the two anterior nuclei lying in the plane of the paper. To reach this position, the axes of the spindles of the two dividing nuclei of the last stage must have rotated in opposite directions. See McMurrich on Isopods for a similar phenomenon (18). All the nuclei are dividing, *d.n.* The chromatin of the polar bodies has become much fragmented, *p.b.*

FIG. 5. Optical section of egg with nine nuclei. Nuclei scattered in yolk. Axes of dividing nuclei of last stage have rotated, as before, to make angles with one another. An odd nucleus shows irregularity in divisions: *p.b.*, polar bodies.

(All the remaining figures of this and the next plate are surface views.)

FIG. 6. Ventral surface of older egg. The cells are at equal distances apart.

FIG. 7. Surface view of right side of egg with more nuclei than the last. The nuclei somewhat more numerous in the posterior half: *p.b.*, polar bodies.

FIG. 8. Ventral surface. Nuclei dividing, *d.n.*, everywhere on surface. Numerous pairs of just separated nuclei, *s.n.*, show division anteriorly as well as posteriorly.

FIG. 9. Ventral surface of egg with twice the nuclei of last. More cells in posterior half, due to movement that way and to multiplication.

FIGS. 10 and 10<sup>a</sup>. Ventral and dorsal surfaces of egg with double the nuclei of last stage. The nuclei of the posterior half of the egg are more numerous than on the other end. They lie rather close together, nearly as far forward as the smaller diameter.

FIGS. 11, 11<sup>a</sup>, and 11<sup>b</sup>. Ventral, dorsal, and lateral surfaces of an older egg. In Fig. 11 of ventral surface, note that the anterior limit of the area *ca.* of relatively closely crowded nuclei of last stage has drawn nearer the posterior pole, away from the smaller diameter of the egg.











Fig. 1.



Fig. 2.

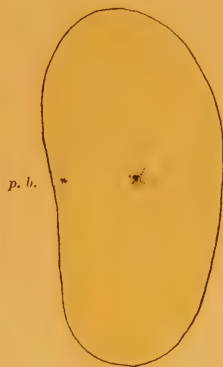


Fig. 3.

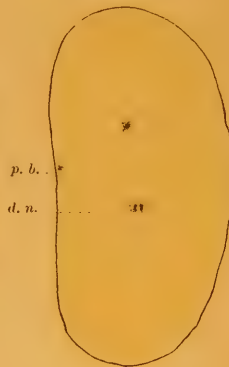


Fig. 4.



Fig. 5.

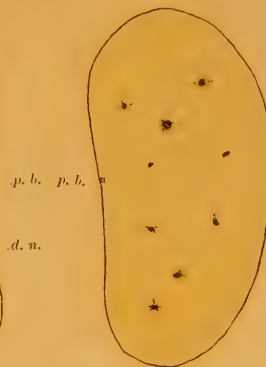


Fig. 6.



Fig. 7.



Fig. 8.



Fig. 9.



Fig. 10.

Fig. 10<sup>a</sup>

Fig. 11.









## EXPLANATION OF PLATE XXX.

FIG. 11a. Dorsal surface. The cells at posterior end of this surface crowded closely together to form posterior border, *p.b.d.* (posterior limit of disc) of the area *ca.* of ventral surface. The collection of nuclei forming this border are in sharp contrast to those scattered over this surface.

FIG. 11b. Side view, giving better idea of how markedly the cells on the surfaces of the egg have crowded back to the region marked *ca.* in figures: *p.l.d.*, posterior limit of cap or disc well shown; *a.l.d.* anterior limit of disc; *A.* and *P.*, anterior and posterior.

FIG. 12. Ventral surface of older egg with disc of nuclei forming area *ca.* about equally distributed to its borders.

FIG. 13. Further contraction of disc; all of its boundaries now well within limits of ventral surface: *l.b.d.*, lateral border of disc.

FIG. 14. Ventral surface of last, seen slightly on one side to show outlines of contracting area.

FIG. 15. Posterior end of egg tipped up to show an especially marked concentration of embryonic disc.

FIG. 16. Ventral surface of older germ-disc torn off with a piece of the chorion: *ch.*, chorion; *ul.p.*, under-layer plug.

FIG. 17. Ventral view of egg showing germ-disc with crowded posterior margin: *am.t.*, amnion thickening, later to fold forward; *ul.p.*, under-layer plug more distinct.

FIG. 18. Ventral surface showing *am.t.*, amnion thickening, at its maximum of crowding before folding over.

FIG. 19. Older egg, somewhat on side to show amnion fold, *am.* The *ul.p.*, extensive under-layer plug. Cells of serosa, and middle thin region of disc, faint.

FIG. 19a. Same stage dissected off to show details. Amnion fold reaches forward on sides anterior to *ul.p.* Anterior limit of disc, *a.l.d.* Note dividing nuclei, straight black rods.

FIG. 20. Side view of amnio-serosal fold, *am.*, soon after its origin. Cells of embryo not shown: *sc.*, serosa cells posterior to fold; *em.d.*, embryonic disc; *sc.a.*, serosa cells on yolk anterior to embryo; *ch.*, chorion.

FIG. 21. Side view of amniotic fold (partly in optical section) half covering the disc: *am.*, amnion, of same appearance as ectoderm of disc, being several layers thick; *am.s.*, side view of amnion; *my.*, two micropyles in distended, wrinkled chorion, *ch.*

FIG. 22. Optical section from side of closing amnion; *o.amc.*, opening from exterior into amniotic cavity; *sc.*, serosal cells, large and faint on surface of yolk; *yc.*, yolk-cells deeply stained and lying within the yolk. Cells of embryo and amnion not shown.

FIG. 23. Same stage as foregoing seen from surface. Lettering as before.





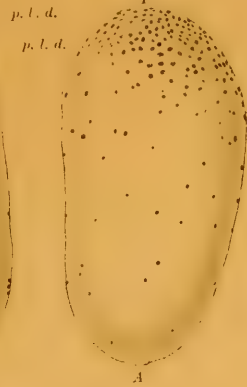
Fig. 11<sup>a</sup>.Fig. 11<sup>b</sup>.

Fig. 12.

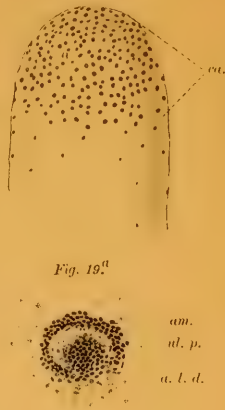


Fig. 13.



Fig. 14.



Fig. 15.

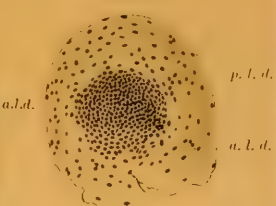
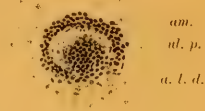
Fig. 19<sup>a</sup>.

Fig. 16.

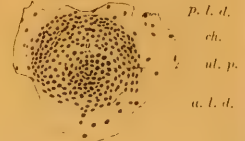


Fig. 22.



Fig. 23.

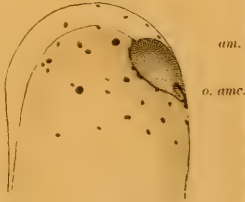


Fig. 17.



Fig. 18.



Fig. 19.



Fig. 20.



Fig. 21.







## EXPLANATION OF PLATE XXXI.

FIG. 24. Ventral surface of egg, just after closure of amniotic cavity: *a.e.*, anterior end of embryo; *yc.*, yolk-cells intensely stained; *sc.*, serosal cells, large and of light color.

FIG. 25. Side view (optical section) of slightly older embryo, slipped back out of its usual position. The black ectoderm appears thicker in such an optical section than it actually is: *y.*, surface of yolk-mass; *a.e.*, anterior end of embryo; *ch.*, chorion; *am.*, amnion; *ul. (mes.)* under-layer, or mesoderm, extending forward beneath ectoderm.

FIG. 26. Ventral view of germ-band two or three stages older than Fig. 24, unsegmented and without cephalic lobes. The germ-band at this age is usually placed as in the next figure.

FIG. 27. Embryo like that in the last figure, in its usual position. Seen from side: *a.e.*, anterior end; *ch.*, chorion; *am.*, amnion; *p.e.*, posterior end.

FIG. 28. Unsegmented germ-band with cephalic lobes, just before the appearance of segments. Same stage as that of next figure, Fig. 29. The embryo is dissected off from the yolk, and drawn with lower (yolk) side uppermost: *am.*, amnion cells, along edges of band; *a.t.*, anterior triangular area, between cephalic lobes. The mesoderm cells are large black masses.

FIG. 29. Unsegmented germ-band in same stage as last. Side view, to show position on yolk. The amnion is faintly seen as a row of small dots beneath the chorion.

FIG. 30. Cross-section through middle of germ-disc, at about the age of Fig. 13, perhaps slightly older: *ul.n. (mes.)* under-layer or mesoderm nucleus crowded below the surface; *d.n.*, nucleus dividing to separate a cell below; *yc.*, yolk cells; *ybs.*, yolk bodies of all sizes, and two perforated with holes left by solution of oil drops. (0.003 mm. thick.)

FIG. 31. Cross-section through region of under-layer plug, *ul.p. (mes.)*, the mesodermal rudiment, at the stage of the germ-disc shown in Fig. 18; *d.n.*, nucleus dividing to separate cell below; *my.i.*, inner opening of micropyle; *my.c.*, penetrating canal of micropyle through chorion; *my.o.*, outer opening of micropylar funnel; *ch.*, chorion; *p.yb.*, perforated yolk bodies. A large yolk-cell lies under the middle of section. Yolk bodies are large. (0.003 mm. thick.)

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The following six sections form a series, illustrating the growth of the germ-disc and the mesoderm; also the origin and growth of the amniotic fold. Nuclei diagrammatic, except the dividing ones.

---

FIG. 32. Median sagittal section of disc at stage in Fig. 18: *am.t.*, amniotic thickening of ectoderm; *ul.p. (mes.)*, under-layer or mesodermal plug; *a.ec.*, anterior end of ectoderm of disc; *yc.*, yolk-cells; *yb.*, yolk body. Four dividing nuclei. (0.004 mm. thick.)

FIG. 33. Median sagittal section of disc, Figs. 19 or 19<sup>a</sup>; *sc.*, serosal cells; *am.f.*, amniotic fold; *ul.p. (mes.)*, under-layer or mesodermal plug; *a.ec.*, anterior ectoderm; *yc.*, yolk-cell. Seven nuclei in various stages of division. (0.004 mm. thick.)

FIG. 34. Median sagittal section of an embryo slightly younger than that in Fig. 22: *sc.*, serosal cells; *y.*, yolk-mass of finely fragmented bodies beneath embryo; *am.f.*, amniotic fold; *o.amc.*, opening into amniotic cavity; *a.ec.*, anterior ectoderm; *ul.p. (mes.)*, anterior end of mesodermal plug; *yc.*, yolk-cell; *p.yb.*, perforated yolk body. Six dividing nuclei are seen. (0.004 mm. thick.)

FIG. 35. Median sagittal section of embryo at stage Fig. 24: *ch.*, chorion; *am.*, amnion; *am.c.*, amniotic cavity, now completely closed; *sc.*, serosal cells; *a.ec.*, anterior ectoderm; *yc.*, yolk-cells; *ul.p.*, mesodermal plug; *mes.*, mesoderm. Seven dividing nuclei in different phases. (0.004 mm. thick.)

FIG. 36. Median sagittal section of embryo, between Figs. 24 and 26, with anterior end square: *sc.*, serosal cells; *s.*, serosa; *am.*, amnion; *am.c.*, amniotic cavity; *a.ec.*, anterior ectoderm; *mes.*, mesoderm; *yc.*, yolk-cell; *yb.*, yolk body. Ten dividing nuclei in different stages. (0.004 mm. thick.)

FIG. 37. Median sagittal section of embryo in Fig. 27 (or 26): *am.p.*, amnion at posterior end; *am.a.*, amnion, thinned out at anterior end; *am.c.*, amniotic cavity; *mes.p.*, mesoderm under posterior end; *mes.*, mesoderm in the middle region; *mes.a.*, anterior limit of mesoderm; *ect.cp.* (anterior ectoderm as in previous figures *a.ec.*), now ectoderm of cephalic region; *p.yb.*, perforated yolk body; *yc.*, yolk-cells, nuclei in large masses of protoplasm. Nine dividing nuclei. (0.004 mm. thick.)











## EXPLANATION OF PLATE XXXII.

The outlines of the figures on this plate were drawn with the aid of a camera. They are magnified 55 diameters by Bausch & Lomb's *oc.* 50 mm., *object.* 17 mm.

The series represents, diagrammatically, in side view and partly in optical section, the principal stages in the development of the Termite embryo, from the complete establishment of the germ-disc to the dorsal closure of the body-walls.

The origin and history of the embryonic membranes are particularly emphasized. The general relations of the embryo to these membranes, to the yolk-mass, and to the axes of symmetry of the egg are also well brought out. Note the position of the micropyles, on the primary (and definitive) ventral surface of the posterior end of the egg, in studying the remarkable changes in position which the embryo passes through.

- 
- STAGE *A.* Germ-disc when first established, *em.*; extra-embryonal blastoderm, *ex.*; yolk, *y.*; chorion, *ch.*; micropyles, *my.*
- " *B.* Germ-disc with posterior amniotic thickening, *am.t.*, and mesodermal plug.
- " *C.* Amniotic fold. Amnion a part of disc, *am.*; serosa, extra-embryonic, *s.*
- " *D.* Amniotic fold just before closure of amniotic cavity. Amniotic cavity open anteriorly, *amc.*; serosa, *s.*
- " *E.* Immediately after closure of amniotic cavity, *amc.*
- " *F.* Early stage in the posterior elongation. The amnion begins to be stretched.
- " *G.* Elongating germ-band before appearance of cephalic lobes. Amnion not fully stretched posteriorly. Mesoderm a flat under-layer.
- " *H.* Unsegmented germ-band with cephalic lobes, *cp.l.*
- " *I.* First appearance of segments. Antennae, *ant.*; mandibular segment, *md.*; first maxillary, *max.*<sup>1</sup>; second maxillary, *max.*<sup>2</sup>; first thoracic, *th.*<sup>1</sup>; tail-piece, *ta.*
- " *K.* Further elongation. Addition of second and third thoracic segments, *th.*<sup>2</sup> and *th.*<sup>3</sup>; and an indistinct first abdominal, *ab.*<sup>1</sup>, from anterior portion of tail-piece. Appearance of labrum, *l.*, and stomodeum, *st.* Folding of head up from yolk.
- " *L.* First stage of caudal flexure. Cephalic and thoracic appendages well marked.
- " *M.* Caudal flexure pronounced. Abdominal segments established. Proctodeum, *pr.*, well developed. Anterior appendages prominent.
- " *N.* Just before "revolution." Head globular and standing off from yolk. Maxillae tri-lobed. Anterior appendages long and beginning to segment. Abdominal appendages prominent. Stomodeum and proctodeum long.
- " *O.* "Revolution." Head slipping up along ventral surface to the anterior pole of the egg. Embryonic membranes, especially serosa, contracting dorsally. Proctodeum, *pr.*, a long tube. Second maxillae moved inward and not seen.

STAGE *P*. Completion of "revolution." Dorsal growth of body-walls. Tracheal stigmata, *tr*. Dorsal organ, *d.o.*, the retracted remnants of embryonic membranes.

" *R*. Closure of body-walls on the mid-dorsal line complete. Dorsal organ has disappeared within yolk-mass, which is now enclosed in mid-gut. Ventral ganglia shown. As compared with the preceding stage, *P*, this is more truly an optical section, not showing the body-walls except along the boundaries of the body.















## THE GASTRULATION OF AMPHIOXUS.

T. H. MORGAN AND ANNAH PUTNAM HAZEN.

MATERIAL for a study of the process of gastrulation of *Amphioxus* was collected in 1895 at Faro, Sicily, and at the Stazione Zoologica in Naples.<sup>1</sup> A part of the material was stained at once and surface preparations made; another part, after staining, was imbedded in paraffine, and the remaining eggs were preserved in alcohol.

Kowalevski's and Hatschek's account of the gastrulation left many points still unsettled, and Lwoff's description of the process was so different from those of his predecessors that the entire problem appeared in a new light. Since our work began, no less than three papers have appeared dealing with the gastrulation of *Amphioxus*. It might seem, under these circumstances, that further work would be superfluous; yet the more we have studied the process in *Amphioxus* the more difficult has the problem appeared, and none of these authors seem to us to have reached a satisfactory conclusion.

### *Methods.*

The eggs were preserved in several ways. Corrosive-acetic preparations are best for surface views, and show very clearly, both in surface views and in sections, the dividing and the resting nuclei. Hermann's fluid blackens the embryo so that it cannot be used for surface preparations. The yolk granules come out very clearly in sections, and the cell boundaries are generally very well shown. Flemming's solution — the stronger formula — gives nearly the same results as Hermann's fluid. Embryos fixed by the two latter solutions do not need subsequent staining, although iron haematoxylin will bring out clearly the nuclei, especially those in process of division. After the

<sup>1</sup> For further details, see Morgan ('96).

corrosive-acetic solution the embryos stain well in picro-lithium carmine. Without using the highest powers of the microscope, very little can be made out of the cell-structure of the embryo. Almost all our work, therefore, has been done with a Zeiss immersion 2 mm.

### *Gastrulation.*

One of the great difficulties in following the changes that take place during gastrulation is due to the absence of landmarks. Our attention has been largely directed toward the discovery of points of orientation of the early gastrula. Hatschek relied mainly on the form of the embryo in optical section, and we have also found this, under certain circumstances, a valuable means of orientation. Wilson noticed that the pore at the vegetative pole, which is sometimes left at the end of cleavage, persists occasionally throughout the early period of invagination, and by this means he showed that the vegetative pole was brought into contact with the animal pole; in other words, that the invagination was radially symmetrical. We hoped at first, by using this opening as a fixed point, to determine the later changes that take place, but all traces of the pore soon vanish, except in very abnormal cases.

It is of importance to determine at as early a stage as possible the orientation of the gastrula; and here we have been more successful, since we have been able to distinguish the dorsal and ventral lips of the blastopore at the beginning of the gastrulation process. The gastrula is not perfectly symmetrical, and the same is probably true of the blastula, although more difficult to demonstrate. During invagination the vegetative pole is brought near the animal pole, yet the endoderm turns in such a way that the dorsal side can be distinguished from the ventral. A careful study of the yolk granules, and their appearance in the cells, has aided greatly in orientation. Certain cells, that are turned in at one side, contain relatively fewer and lighter yolk granules, and these cells can be traced from the first stages of invagination until the closing of the blastopore. They mark the dorsal side. As this is a constant feature, it gives a definite means of orienting the embryo.

We have also studied the number and position of the cells surrounding the blastopore at different periods, in the hope of discovering some region of more rapid growth. Finally, the karyokinetic phenomena of the embryo have been carefully examined, for on this process Lwoff has mainly relied. It has been necessary to take all these factors into consideration in order to follow the changes that take place during gastrulation.

Kowalevski and Hatschek have shown that the blastula is composed of larger and smaller cells, and several more recent authors have observed the same fact. MacBride makes the surprising statement that the cells of the blastula wall are all of equal size. He has probably confused cross-sections of the blastula with longitudinal ones, otherwise it is difficult to see how such a mistake could have been made.

The yolk granules almost completely fill the cells around the vegetative pole and gradually diminish in number toward the animal pole.

The gastrulation begins by a flattening of the vegetative part of the egg (Pl. XXXIII, Fig. 1). The lower hemisphere then turns in and slowly obliterates the segmentation cavity (Pl. XXXIII, Fig. 2). The invagination is not entirely radially symmetrical, for the inturned cells bend over more toward one side of the embryo than toward the other (Pl. XXXIII, Fig. 2). At this time the segmentation cavity almost disappears at one side, which becomes subsequently the dorsal side of the embryo, but a portion of the segmentation cavity is left around the remaining part of the inturned cell plate, and, in general, is largest exactly opposite the dorsal side, and in this way a dorsal and a ventral side of the gastrula are distinguishable at an early stage of gastrulation. The outline of the embryo may also be used, as Hatschek demonstrated, to determine points of orientation, as shown in Pl. XXXIII, Fig. 2. The outline of the dorsal side is less rounded than that of the ventral side. This seems to be constant; but, if the sections do not pass dorso-ventrally, this difference cannot be made out.

A further point of orientation is found in the appearance of the inturned cells of the dorsal side. These cells are somewhat smaller than the other invaginated cells, and they resemble

closely the cells of the outer surface of the dorsal side (Pl. XXXIII, Fig. 1). On the other hand, at the ventral lip there is rather an abrupt transition between outer and inner cells (Pl. XXXIII, Fig. 2). MacBride has called attention to these differences, but it is not improbable that in the early stages, at least, he has confused the dorsal and the ventral sides of the gastrula.

Although the embryos can be oriented, as stated above, yet it is still very difficult to determine how the closure of the blastopore is brought about. While the blastopore becomes smaller, the embryo is, at the same time, changing its shape, so that it is not possible to assume that any one point is fixed in relation to the others. Moreover, the possibility of cell-migration must also be kept in mind. Lwoff has considered the presence of karyokinetic division as a criterion of growth; but it must not be forgotten that growth need not follow unless after division the cells increase in size. Furthermore, cell migration is known to take place without cell division.<sup>1</sup>

As stated above, we had hoped to make use of the vegetative pore, and by this means to determine how the closure of the blastopore takes place. The pore is most often present in series of eggs that do not develop normally, and hence there is a certain amount of risk in using such a feature to determine the changes that take place in the normal egg. The pore seems generally to disappear in the later stages. Moreover, other openings are sometimes found in the endoderm, due, in some cases at least, to changes in shape of the cells at the time of division. Pl. XXXIV, Fig. 17, shows a cross-section of an egg in which the vegetative pore is large, and lies near the highest point of the invaginated cells.

Occasionally a gastrula is found with the cells around the vegetative pore turned outward (Pl. XXXIV, Fig. 16); and the cells remain in this position even during later stages, as shown in Pl. XXXIV, Fig. 19. This embryo throws some light on the way in which the blastopore closes (Pl. XXXIV, Fig. 19). As it was found among embryos in which, on an average, the

<sup>1</sup> In the sea-urchin the archenteron is formed by cells pushing into the segmentation cavity.



blastopore was nearly closed, the figure shows the relative growth of the dorsal and ventral walls. It will be noticed that the ventral wall is longer than the dorsal, and this is confirmed by other results.

In one case that we have met with, an ectodermal pore was present at the animal pole (Pl. XXXIV, Fig. 18), yet the presence of this pore has not prevented the invagination of the larger cells. If, as seems probable, the opening was present during the gastrulation period, the embryo shows that the process of gastrulation may take place even when a large pore is present in the wall. That this is possible is also shown when the vegetative pore is present, and yet invagination takes place. Therefore, whatever mechanism is invoked to explain the process of gastrulation, the process is of such a nature that it does not demand a closed blastocoel space.

At first the outline of the blastopore is oval (Pl. XXXIV, Fig. 11). A large number of cells bound the opening, but, as there is no sharp line of demarcation between the outer and the inner invaginated cells, and since some of the cells at the edge are partly within and partly without the rim of the blastopore, the exact number and the shape of the boundary cells cannot be accurately determined. In Pl. XXXIV, Fig. 11, about forty-two cells form the rim of the blastopore. At a later stage the number is smaller. In Pl. XXXIV, Fig. 12, about thirty-four cells are around the margin. When the blastopore has further closed (Pl. XXXIV, Fig. 13), twenty-eight cells were counted. Finally, when the blastopore is reduced in size, as shown in Pl. XXXIV, Fig. 14, only ten cells were present.

How can we picture to ourselves the gradual reduction in number of the cells as the blastopore becomes smaller? It appears at times that, as the rim of the blastopore diminishes, certain of the cells continue at the edge, but that others are left behind in the general movement toward the center. In the latest stage the boundary cells are elongated, and as a result a larger number of cells surround the reduced blastopore than would be the case if the cells all retained their earlier form. If we judge by the shape alone of the blastopore, the closing takes

place at nearly the same rate from all points; at least, until the blastopore is much reduced in size. The outline of the blastopore is generally oval, with the long axis connecting the dorsal and ventral sides. At the time when the embryo is flattened on the dorsal side to form the nerve plate, the blastopore changes its shape, so that it becomes somewhat elongated from side to side (Pl. XXXIV, Figs. 13 and 14).

Karyokinetic figures are found sometimes parallel to the margin (Pl. XXXIV, Fig. 12), sometimes at right angles to it. There is great irregularity in the distribution of the dividing cells. In some embryos a large number of cells are in process of division, in other embryos nearly all the cells are in a resting stage. Sometimes groups of cells may be found dividing and others resting, but such occurrences are exceptional, and, as a rule, the karyokinetic figures are scattered irregularly over the surface.

Lwoff has claimed that at a certain period cell division is more rapid on the dorsal side of the blastopore than elsewhere, and, in consequence, the ectoderm turns in and forms the dorsal wall of the archenteron. A careful examination of a large number of embryos at all stages of development gives no support to this view.

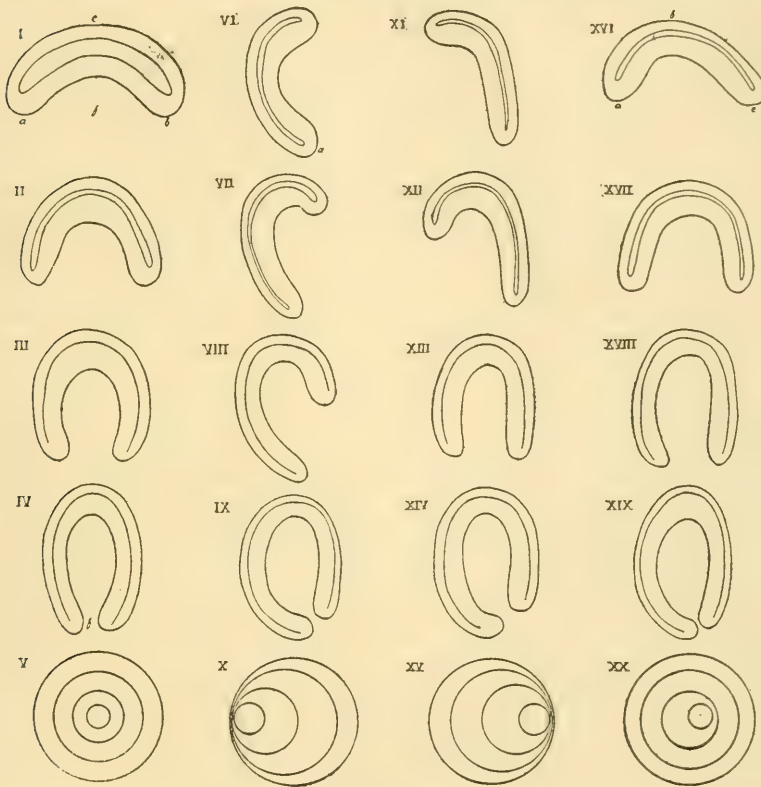
It is important in comparing the changes in the shape of the embryos of different stages to use the same series preserved by the same methods. It is also important that sections in the dorso-ventral plane and in oblique planes be carefully distinguished.

The early gastrula is shallow and saucer-shaped (Pl. XXXIII, Fig. 2). The lips are then brought nearer together, so that the embryo is longer, but correspondingly narrower (Pl. XXXIII, Fig. 3). The embryo continues to narrow dorso-ventrally and also laterally, and at the same time it grows longer. This process continues, and for a time the growth of the dorsal and ventral lips seem to be about equal. In still later stages (Pl. XXXIII, Fig. 5) the ventral wall seems to grow more rapidly; it bends inward toward the dorsal surface, and extends farther posterior than does the dorsal lip.

Since there are no definitely fixed points, it is very difficult

to determine in what region the bending in of the wall takes place. The method of closing cannot, therefore, be determined by any one criterion, but all the observed results must be taken into account.

It should be noticed that in these figures the dorsal (*d.*) and the ventral (*v.*) lips of the blastopore are, throughout the early



FIGS. I-XX.

stages, equally distant from the point of greatest curvature (*a*). If, on the other hand, the bending took place nearer the dorsal or the ventral lip, one side would be shorter and the other longer; and this is not the case.

Hatschek figures one side — the ventral — as longer than the other side, — the dorsal, — but the dorsal subsequently grows in length until it is approximately the same length as

the ventral; the ventral wall increasing very little, if at all, during the period of closing. A comparison of our Figs. 2, 3, and 5, Pl. XXXIII, shows unmistakably that both the ventral and dorsal walls are longer in the older stages.

A comparison of Hatschek's Fig. 24 with our Pl. XXXIII, Fig. 2, will show that what Hatschek supposed to be the dorsal side is in reality the ventral one.

It is important to keep in view the different ways in which the gastrulation might be interpreted, and we have tried to illustrate in the accompanying text-figures the different possibilities. The first series, I-V, represents a symmetrical closure of the blastopore. The final closure at *f* is opposite the anterior end *c*. The primary axis of the blastula and of the gastrula corresponds with the antero-posterior axis of the embryo. The closure of the blastopore is shown in V, and is seen to take place equally from all points. Kowalevski supposed this to be the way in which the blastopore closes.

The second series, VI-X, represents an unsymmetrical closure of the blastopore. The ventral lip at *a* is supposed to be fixed in VI, and in the successive figures, VII-IX, the dorsal lip is represented as bending over and elongating to close the blastopore. The closure of the blastopore takes place over the dorsal side of the embryo. Fig. X shows the successive stages in the closure of the blastopore. If we compare Figs. V and X, we see that when the blastopore is turned upward toward the observer the point of greatest bending of the wall is opposite the blastopore in V, while in X the point of greatest bending is anterior to the center of the blastopore (see also Fig. VIII). This series, VI-X, represents Hatschek's idea as to the way in which the blastopore closes.

The reverse method of closing is shown in series XI-XV. Here the dorsal lip is represented as fixed, and the ventral as bending over to close the blastopore. Hence the closing is on the ventral side of the embryo.

It will also be noticed that in the last two series (VI-X and XI-XV) the point of greatest bending shifts gradually around the anterior end of the embryo as the closing takes place; while in the first series the point of greatest bending remains fixed.











There are, in fact, two ways in which we may think of the changes shown in Figs. VI-X and XI-XV as taking place. The wall that grows backward to close the blastopore may either simply elongate as a result of cell growth, and the bending at the anterior end remain fixed, or the growth backwards might be due to a gradual shifting of the part of greatest bending along the opposite side; in consequence of this the dorsal wall would increase in length at the expense of the ventral in one case, and the ventral at the expense of the dorsal in the other. It is also possible that both a change in the bending and cell growth might take place at the same time.

In a fourth series we have tried to represent our own idea of the method of closure of the blastopore. In the first figure of this series, XVI, the gastrula is represented as being somewhat unsymmetrical. The dorsal lip is at *c*, the ventral at *a*. The point of greatest bending of the gastrula is near the center, but a little toward the ventral side. At a later stage, XVII, both dorsal and ventral sides of the gastrula have come nearer together, and the embryo has, in consequence, become longer. The dorsal and ventral sides are of about equal length, and the point of greatest bending is opposite the blastopore. In a later stage, XVIII, the walls grow longer and the dorsal side flattens somewhat. The opening of the blastopore is still opposite the point of greatest bending. In the last stage, XIX, the ventral lip grows faster and the blastopore opens more on the dorsal side. Fig. XX shows the method of closure as seen from the posterior side. The series of concentric circles represent the successive stages. The final stage is excentric, owing to the more rapid growth of the ventral lip.

If instead of a dorso-ventral series we had made use of a lateral one, that is, one from right to left, to illustrate our idea of the method of closure of the blastopore, we would have given a series exactly like that drawn in I-V. Even in the dorso-ventral series our idea of the method of closure corresponds more nearly to that of the first series than to any other; the points of difference being: first, the unsymmetrical gastrula at the earliest stage (XVI); second, the flattening of the

dorsal side in XVIII; and, third, the more rapid growth of the ventral wall in the final stage of closure.

*Cell Division during Gastrulation.*

Cell division occurs throughout the entire period of gastrulation both in the ectoderm and in the endoderm. We have examined a large number of preparations, both sections and surface views, to see whether cell division is more frequent in one part of the embryo than in another. Although cell division is present at all times and in all stages, yet we have found, in general, no region of more rapid cell multiplication.

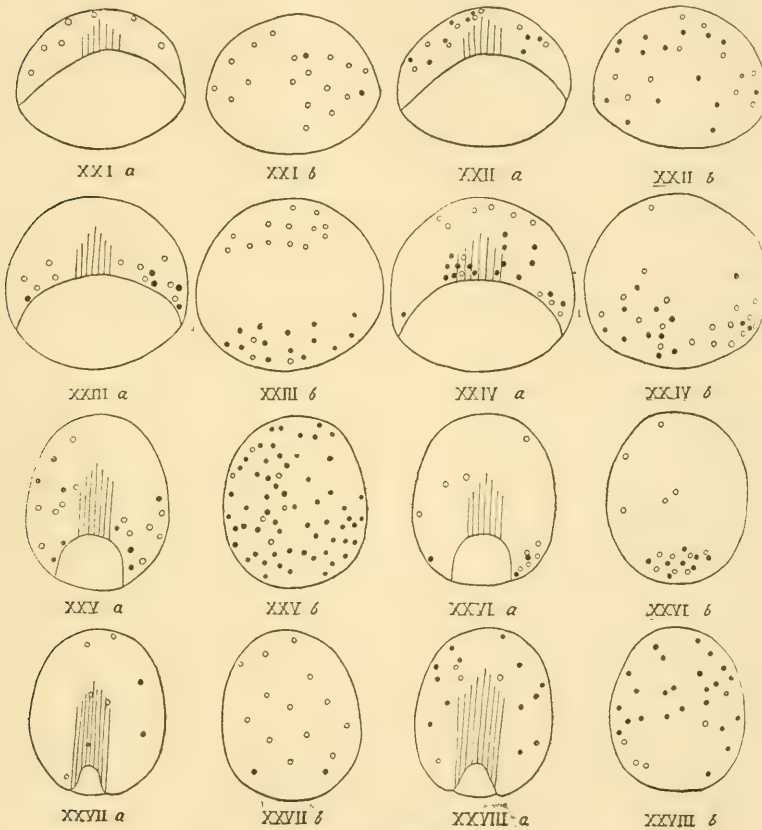
Great individual differences exist, for while one preparation may show more karyokinetic figures on the dorsal or on the ventral lip of the blastopore, other preparations of the same age may show other regions of cell multiplication. Hence, with a preconceived idea as to the place at which more rapid growth occurs, one could easily find preparations confirming such a view. But an unprejudiced examination shows that cell division is general and not restricted to any special region. Text-figs. XXI-XXVIII show in what regions cell division is taking place in certain embryos of various ages. The drawings are diagrammatic reconstructions of serial sections. The dorsal side is indicated by the parallel lines. One figure of each pair shows, therefore, the dorsal, and the other the ventral side. The dividing nuclei in the ectoderm are represented by solid dots, and those in the endoderm by circles.

Fig. XXI *a, b* represents the embryo at midnight, corresponding in age with the embryo shown in Pl. XXXIII, Fig. 2. Only two ectodermal cells are dividing, while many endodermal cells are in process of division. Another embryo of the same age is shown in Fig. XXII *a, b*. Here the number of cells dividing in ectoderm and endoderm is about the same. The dividing cells are scattered nearly equally throughout the embryo.

The next stage, Figs. XXIII and XXIV *a, b*, represent the embryo at 3.30 A.M. Both show cells dividing around the blastopore, and there is also a region of division at the anterior end.

In one of these the ectoderm cells on the dorsal side and in the other on the ventral side are dividing more rapidly. Other preparations of the same age were found in which the cell division is not more common around the blastopore than elsewhere.

At 4.30 A.M. the embryo has closed in further (Figs. XXV *a*, *b* and XXVI *a*, *b*). In the first of these figures it is



FIGS. XXI-XXVIII.

noticeable that cell division is very frequent in the ectoderm of the ventral side. In the other very little cell division is present, and that mostly around the ventral lip of the blastopore.

Figs. XXVII *a*, *b* and XXVIII *a*, *b* at 6 A.M. show, in one case, several endoderm cells and few ectoderm cells in process of division. In the other embryo the reverse is true.

After this stage the blastopore closes in more rapidly from the ventral side, and cell division seems to be more rapid on this side, both in ectoderm and endoderm.

*Distribution of Yolk in the Embryo.*

At the time when the blastula wall first begins to flatten we have attempted to discover whether the yolk is equally distributed throughout the endodermal plate. In many cases we have found that the embryos preserved in Flemming's or in Hermann's fluid show at one end a region of small cells bearing fewer yolk granules, and these granules are lighter in color than those of the rest of the inturned cells<sup>1</sup> (Pl. XXXIII, Fig. 1). At the opposite end of such a section there is an abrupt demarcation between the large yolk-bearing cells that are turned in, and the smaller and lighter cells outside. This difference in the two ends of the section is shown by many series. In other series, cut more or less at right angles to the preceding, the difference is not seen, of course, in the middle sections of the series, but generally at one end of the series the lighter cells can be found, and at the opposite end the yolk-bearing cells extend to the margin of the blastopore.

This difference in the endoderm we have been able to trace throughout all the subsequent periods of development, and we have been able to show that the region of clearer cells becomes the dorsal wall of the archenteron. Hence the dorsal lip of the blastopore is identified even at the time when the first beginning of gastrulation is evident. It is, therefore, highly probable that even in the spherical blastula there is a bilateral symmetry already present. We have laid more stress on this means of orientation than on any other, and have determined the dorsal and ventral sides in this way.

A later stage in the gastrulation is shown in Pl. XXXIII, Fig. 2. At this time the endodermal plate has bent upward and almost touches the ectoderm. The section is in a dorso-

<sup>1</sup> It is difficult to determine whether the lighter granules are in reality lighter than those elsewhere, or whether it is only an optical effect of the light passing through fewer granules.



ventral plane and shows the difference in the ectoderm at the dorsal and ventral lips of the blastopore. At the dorsal lip the endodermal cells are, as before, lighter and contain less yolk and differ little from the ectodermal of the outer surface; in fact, there is really no histological difference between the cells just outside and just within the lips of the blastopore over the dorsal side. It is to be noticed that there are more of the lighter cells on the dorsal wall than in the preceding stage. This might result either by a continued rolling in of outer cells, or by a multiplication of cells already inside, or by both processes combined, or even by differentiation of the cells anterior to the clear region. It is certain that the endodermal cells continue to divide during development, and we have seen no evidence of an actual inrolling. Whether the division of the cells in this region is, in itself, sufficient to account for the increase in the number of cells is a point almost impossible to determine.

The same section (Pl. XXXIII, Fig. 2) shows also the characteristic shape of the embryo at this stage; over the dorsal side the embryo is less curved than over the ventral side. The deepest part of the invaginated endoderm is somewhat toward the ventral side. The effect produced is that the ventral part of the endoderm is thrown over toward the ventral side of the embryo. Surface views of entire embryos show the same asymmetry in the gastrula. On the ventral side of this section (Pl. XXXIII, Fig. 2) there is an abrupt transition between the large yolk-bearing cells and the smaller cells that continue out into the ectoderm. Whether these smaller cells that are partly turned into the blastopore belong to the endoderm or to the ectoderm can only be determined by their fate. They will be considered later.

There is generally present on the ventral edge a dilatation of the segmentation cavity that is characteristic for this period; on the dorsal side the ectoderm and the endoderm are almost in contact.

An older stage is shown in Pl. XXXIII, Fig. 3. The embryo is now cup-shaped and the archenteron much deeper than before. The deepening is, in part, the result of the

approach of the sides of the embryo, so that it is now narrower dorso-ventrally and also from side to side. As a result, the blastopore becomes smaller (Pl. XXXIV, Figs. 11 and 12) and at the same time the saucer-shaped embryo (Pl. XXXIII, Fig. 2) becomes elongated into a cup-shaped form. The cells are smaller and more numerous in the older embryo (Pl. XXXIII, Fig. 3), but there is no evidence that the change in form of the embryo is the result of cell activity in any particular region. The dorsal wall of the archenteron is covered by lighter cells, and this region is longer than in the preceding stage. The clear cells of the dorsal wall are now larger than the ectodermal cells outside the blastopore, although just at the lips of the blastopore the transition between inner and outer cells is gradual. At the ventral lip the yolk-bearing cells come nearly to the lip of the blastopore and are followed by a few small, rounded cells. The largest endodermal cells bearing the greatest number of yolk granules are found at the innermost part of the archenteron; the cells decrease gradually in size, and in the number of granules contained in them, toward the ventral side.

Around and within the ventral lip of the blastopore, during the early gastrula stages, there are frequently found, as just described, small cells, that contain much less yolk than the large endodermal cells, and closely resemble the ventral ectoderm in appearance (Pl. XXXIII, Figs. 2 and 3). Even in later stages these small cells are often found. We have carefully examined many different series of sections to see if the spherical form of these cells is the result of cell division, but cell division does not seem to be more frequent here than elsewhere, and the nuclei of the cells are often in the resting stage. It is difficult to determine whether any of these cells come to lie eventually in the archenteron, or whether in later stages, as the yolk-bearing endodermal cells multiply, these cells turn out into the ectoderm.

The dorsal side of an embryo (Pl. XXXIII, Fig. 4 A) somewhat older than the last is shown in Pl. XXXIII, Fig. 4 B. The lighter cells of the dorsal wall are shown also in the figure, and their similarity to the ectoderm outside is evident. The

yolk granules in the ectoderm are fainter and are beginning to disappear. The granules grow fainter in that part of the ectoderm which corresponds to the animal pole. Around the blastopore they persist longer; the cells outside the dorsal lip containing granules similar to those in the cells of the dorsal wall of the archenteron.

An older stage, two hours later than the last, is drawn in Pl. XXXIII, Fig. 5. The embryo has greatly elongated, the blastopore is reduced in size and now opens somewhat on the dorsal surface at the posterior end of the body. The cells over the dorsal wall of the archenteron are broader than those elsewhere; they are also clearer and contain fewer and lighter yolk granules. As much as two-thirds of the dorsal wall is formed by these cells, which pass gradually into the more anterior cells containing more yolk. The cells of the ventral wall are smaller than those at the anterior end and are also smaller and darker than those over the dorsal wall.

The cells of the ectoderm over the dorsal surface are very similar to the endoderm cells over the dorsal wall. The more anterior of those ectodermal cells are smaller, while those near the dorsal lip of the blastopore are almost identical with those within.

On the ventral wall the ectodermal cells are smaller than elsewhere, but they grow larger as they approach the ventral lip of the blastopore. At the sides of the blastopore, also, there are larger cells, so that a ring of large cells surrounds the blastopore.

In the later stages the cells over the dorsal and dorso-lateral walls of the archenteron continue to grow clearer and the yolk in them to disappear. A cross-section of an embryo at 8 A.M. is shown in Pl. XXXIII, Fig. 6. Here the difference between the dorsal and ventral wall is clearly seen. The dorsal surface of the embryo is slightly flattened to form the medullary plate. This section is taken at about the level of the posterior third of the embryo. Sections of the same series taken at the extreme anterior end show that the cells on the dorsal wall contain more yolk and resemble those on the ventral wall.

In the later stages changes take place in the endoderm, bringing about a rearrangement of the cells and also involving

a change in the character of the cells themselves. The anterior end of the archenteron becomes larger, and at the same time the cells covering this portion of the cavity become smaller (Pl. XXXIII, Fig. 7 *A*). This region was at first surrounded by the large dark yolk-bearing cells (Pl. XXXIII, Fig. 5), but these cells seem to shift posteriorly; at any rate, the large yolk-bearing cells are now found on the ventral and ventro-lateral walls of the archenteron at the posterior end of the body.

About this same stage, or a little later, a curious change takes place that has greatly puzzled us. Yolk granules begin to appear in all the cells of the body, and what is most surprising is that the yolk in the large endodermal cells does not seem to decrease in amount. We cannot offer, therefore, any explanation as to the meaning of this phenomenon. It might seem that the change was brought about by a decrease in the volume of the cells, and that they contained the same absolute amount of yolk as before, the change being apparent rather than real. Measurements of the embryo show, however, that it is increasing in size, and the body-walls are as thick or thicker than before. The cells are, it is true, higher and narrower than before, but we do not see how this alone could produce the effect. However, even in the cells of the ectoderm yolk granules reappear in later stages. A longitudinal section of an embryo at 11 A.M. is drawn in Pl. XXXIII, Fig. 7 *A*. A detailed and more magnified part of the dorsal wall (at *x*, in Fig. 7 *A*) is shown in Fig. 7 *B*. The ectoderm that forms the nerve plate contains more yolk granules than in the earlier stages, and the endoderm of the dorsal wall (*end.*) is now also filled with yolk granules. Another part of the same embryo anterior to the last (at *y* in Fig. 7 *A*) is shown in Fig. 7 *C*. In this region the ectoderm contains almost no yolk, but the endodermal cells are filled with it. A section through the ventral wall (at *z* in Fig. 7 *A*) is shown in Fig. 7 *D*. Here the ectoderm contains very little yolk, while the endodermal cells are tall, closely packed together, and filled with yolk granules.

A later stage (Pl. XXXIII, Fig. 8) shows that the yolk granules reappear even in the ectoderm. At this time the first













eight body cavities are given off; the cells that form the notochord, and the mesodermal pouches are filled with yolk granules, and the ectoderm is also. This reappearance of the yolk in the later stages occurs after the blastopore has been closed, and at a time when all the regions of the body are easily distinguishable. What we have stated in regard to the earlier stages, throughout which the yolk has been gradually disappearing from certain cells and remaining constant in others, is not effected by these later changes. We feel, therefore, no less certain of our results, based, as they are, largely on the distribution of the yolk and the histology of the cells during the early stages.

*Irregularities in the Endoderm.*

In looking for the vegetative pore as a means of orienting the gastrula stages, we soon found that, at other places, depressions occurred in the endoderm resembling the point at which the vegetative pore closes (Pl. XXXIII, Fig. 9). We had, therefore, to abandon this landmark as a means of orientation. Often we have found that irregularities in the endoderm are due to the rounding up of cells during the final stages of division. In other cases, as in Pl. XXXIV, Fig. 20, at *x*, the nuclei are in a resting condition, but this may be interpreted to mean that after the division the nuclei have become spherical, but the cells have not yet lost their rounded contour. This view is supported by the fact that two such cells are generally found together. In the ectoderm, too, we have often seen two dividing cells showing the same tendency to become spherical during division. However, cells in all phases of division may be found that have not rounded up as described, yet even in these cases the inner free ends of the cells are rounded, thus breaking the even contour of the endoderm.

*Oblique Sections through Blastopore.*

It has been pointed out that the early gastrula is somewhat asymmetrical (Pl. XXXIII, Fig. 2), the invagination being

deeper on the ventral side, making the outline of the embryo more rounded there. A somewhat similar effect is sometimes produced by an oblique section through a slightly elongated embryo. Sections similar to text-fig. XXIX *d* are sometimes obtained, but it is unsafe to judge from such a section alone that the more rounded side is the ventral one. The entire series of sections must be known, *a-f*. During the first stages of gastrulation there is an extraordinary amount of variation in the shape of the embryos. Forms with a somewhat elongated blastopore are not infrequent, and an oblique section of such an embryo produces the effect shown in the figures. Even in the later stages, the same asymmetry may be found as shown in Pl. XXXIII, Fig. 4 *A*. As a matter of fact, the dorsal side of the embryo is in this case the shorter



FIG. XXIX.

one, but other embryos of the same age may be cut in such a way that the shorter side is the ventral one. It would therefore be easy to arrange a series of these forms in such a way as to make it appear that the closure of the blastopore is over the dorsal side. Only after a careful and prolonged examination of both sections and surface views have we been convinced that this condition is the result of an oblique section of a somewhat elongated embryo. If, for instance, the series of sections to which XXIX *c* belongs, be examined, it will be found that at one end of the embryo the sections are completely closed, *a-b*; but at the other end of the series the opening of the blastopore enlarges, as shown in *e, f*.

Sections of the kind, with the dorsal side shorter than the ventral, might seem to confirm Hatschek's view of the closing of the blastopore over the dorsal side, but we hope we have shown this view to be erroneous. Without other means of orientation the shape of the section may be very misleading,

especially when such great variation is present. Sections of embryos preserved in corrosive acetic will not show the differentiation of the dorsal wall upon which we have mainly relied, and after repeated examination of series of sections preserved by this fluid, we have been obliged to give up all hope of orienting with certainty the early gastrula stages. In the later gastrula stages the shape of the embryo is sufficiently characteristic to determine its orientation, but, in the early stages, while in many cases the shape of the embryo might seem to be sufficiently characteristic to determine the dorsal and ventral sides, yet such a criterion alone is very unsafe. We do not hesitate to say that many of the sections figured by some of the more recent authors are in all probability oriented wrongly.

#### *Historical Review.*

Kowalevski gave a very brief account of the process of gastrulation in *Amphioxus*. A radially symmetrical invagination is described; the gastrula grows longer, and the small blastopore lies at the posterior end. Later the blastopore shifts somewhat to the dorsal side. More recent writers have inferred from Kowalevski's brief account that the gastrula axis corresponds to the embryonic axis, and while such a conclusion is probably true, yet Kowalevski's description is so very brief that we can only infer this to be his meaning.

Hatschek claimed to have been able to distinguish a bilateral symmetry at the time when the invagination is completed. We have shown that even at an earlier stage the bilaterality is present and is shown by the differentiation in the flat plate that is subsequently turned in. Hatschek noticed that the large yolk-bearing cells around the vegetative pole of the blastula, that subsequently form the endodermal plate, occupy only about one-third of the circumference of the blastula wall, and hence are, at first, too small to fill the entire inner surface of the cap-shaped gastrula. He supposes that increase in the volume of the endodermal cells takes place during invagination, and this increase, he suggests, is brought about by absorption of the fluid of the blastocoel space; yet his figures show more

large yolk-bearing cells in the endoderm after invagination than in the flattened endodermal plate of the blastula. Lwoff points out the insufficiency of the mechanism proposed by Hatschek and claims that other cells beside the large yolk-bearing ones are also turned in, and in this way he accounts for a sufficient number of cells to fill the archenteric cavity. We have also tried to show that, at the dorsal side, cells poor in yolk are invaginated, although we prefer to speak of these cells as endodermal and not as ectodermal, as Lwoff has done.

Hatschek's idea of the method of closure of the blastopore is illustrated by our series of text-figures, VI-X. The dorsal lip is supposed to bend around and meet the ventral lip, thus closing the gastrula mouth along the dorsal side of the embryo. Hatschek offers this view, not in a dogmatic spirit, but simply as more in line with his own observations, admitting, however, that Kowalevski's view may be the correct one. Hatschek noticed the early asymmetry of the gastrula, but a comparison of his Fig. 24 with our Pl. XXXIII, Fig. 2 shows that what he has identified as the dorsal (anterior) lip of the blastopore is, in our estimation, the ventral lip. The older stages, however, are oriented in the same way as are our own. Hatschek noticed that the transition from ectoderm to endoderm is sharpest on the ventral side, and at that point in the later stages he located the two historic pole cells.

It is not entirely clear to us how Hatschek imagined the backward growth of the dorsal lip of the blastopore to take place. His figures lead us to suppose that the result is, in part at any rate, produced by an increase in the number of cells of this region. The ventral wall remains unchanged, and the ventral lip bends around only so far as it takes part in the reduction of the blastopore.

Lwoff draws a sharp distinction between ectoderm and endoderm; the latter cells being characterized by their size and the amount of yolk contained in them, and he believes that the difference is present even in the blastula stage, so that "ehe die Einstülpung beginnt, dass also die Sonderung der zwei primären Keimschichten — des Ektoderms und Entoderms —



hier als Resultat der Furchung zu betrachten ist."<sup>1</sup> He believes the difference has an important practical meaning, for when the invagination is completed, not only the endoderm, but also a portion of the ectoderm is turned in, so that, in the gastrula of *Amphioxus*, not all the cells that line the archenteron are to be designated as endoderm.

Lwoff dissents from Hatschek's statement that at the end of cleavage and before the gastrulation begins all division ceases. On the contrary, Lwoff points out that cell division continues throughout the gastrulation period,—as we have also found,—and that the ectodermal cells divide more frequently than do the endodermal. Lwoff further differs from Hatschek as to the way in which gastrulation takes place. Hatschek states that the endodermal cells are turned in to form the inner layer of the cap-shaped stage and line the entire inner cavity of the cap. Lwoff thinks that the invagination continues during the period of closure of the blastopore. The increase in the ectoderm cells is, according to Lwoff, a most important factor in the gastrulation process. "Auf den Längsschnitten durch die Gastrula sieht man Mitosen überall im Ektoderm, am zahlreichsten aber sind sie an der Seite, die später zur Rückenseite der Larve wird und am dorsalen Umschlagsrande zu bemerken. Man sieht Mitosen auch in den Zellen der dorsalen Wand der Höhle, die sich als eingestülpte Ektodermzellen erweisen. Die Längsschnitte zeigen, dass die Ektodermzellen an diesem Umschlagsrande umbiegen und nach innen wachsen. Das Anwachsen der Zellen muss an dieser Stelle sehr bedeutend sein, weil auf Medianschnitten der Gastrula sich eine Anhäufung von Zellen oftmals bemerken lässt; die Zellen verlieren hier den Charakter des einschichtigen Epithels und sind unregelmässig zweischichtig gelagert. Manchmal lassen sich an der dorsalen Wand der Höhle Unebenheiten bemerken und einzelne Zellen lösen sich sogar aus dem Zellverbände los und erscheinen als rundliche Zellen, die neben den übrigen Zellen liegen. Ich muss hervorheben, dass die Umbiegung der Ektodermzellen und deren Einstülpung nur am dorsalen Umschlagsrande sich bemerken lässt, am ventralen Umschlagsrande dagegen eine scharfe Grenze

<sup>1</sup> Lwoff, p. 5.

zwischen den Entoderm- und Ektodermzellen sichtbar ist. Man könnte freilich den Einwand erheben, dass alle eingestülpten Zellen als Entodermzellen zu bezeichnen sind; aber dieser Einwand könnte nur auf der vorgefassten Meinung beruhen, dass alles, was nach innen gelangt, als Entoderm zu bezeichnen ist. Ich habe schon oben in Uebereinstimmung mit Hatschek angegeben, dass der Unterschied zwischen den Ektoderm- und Entodermzellen schon im Blastulastadium, also vor der Einstülpung, sich bemerken lässt; dieser Unterschied kann seine Bedeutung nicht verlieren, wenn es sich ergibt, dass die Ektodermzellen sich an der Einstülpung auch betheiligen. Die aktive Rolle der Ektodermzellen bei der Einstülpung und die Betheiligung derselben an der Bildung der dorsalen Wand der Höhle kann auf solche Weise keinem Zweifel unterliegen."<sup>1</sup>

In regard to the mechanism of the invagination, Lwoff again dissents from Hatschek's view. He shows that Hatschek's account of the enlarging of the endodermal cells is an insufficient explanation in itself, and, moreover, the inturned cells, instead of enlarging, are reduced in size by cell division. We have found that it is difficult to tell how much the endodermal cells increase in volume after each division. A very slight increase in the size of each cell would be sufficient to greatly increase the area of the cell plate, even if the cells themselves do not after division assume their original size. Lwoff's statement that during gastrulation the endodermal cells almost cease to divide is certainly incorrect. Dividing cells are to be found throughout the entire gastrulation period in the endoderm. Some preparations even show only the endodermal cells dividing, and others only the ectodermal. Lwoff has observed the latter only, but an examination of a large number of embryos shows that division takes place in both layers. The number of ectodermal cells is larger than that of the endoderm, so that cell division might be found somewhat more often in the outer layer; but from this it would not follow that any cell of the ectoderm divided more often than any cell of the endoderm. Further, Lwoff's statement that cell division is more abundant on the dorsal side of the blastopore is certainly incorrect, since

<sup>1</sup> Lwoff, p. 7.

our preparations show that cell division is no more frequent here than elsewhere. Lwoff attempts to explain the gastrulation as a result of the more rapid division of the ectoderm at the dorsal lip of the blastopore: "Die Einstülpung beginnt an der Grenze zwischen den Ektoderm- und Entodermzellen, wo der Unterschied zwischen Wachstumsenergien beider Elemente am grössten ist. Da aber die Zellenvermehrung nicht überall gleichmässig vor sich geht, sondern sich vorzugsweise an einer Seite konzentriert, die zur Dorsalseite der Gastrula wird, so erklärt sich dadurch die Ungleichmässigkeit der Einstülpung und die Entstehung einer radial-unsymmetrischen Gastrula. Während nämlich an anderen Stellen die Entodermzellen eingestülpt werden, stülpen sich an dieser Seite die Ektodermzellen selbst nach innen ein. Mit anderen Worten, die Zellen, die vom dorsalen Umschlagsrande aus nach innen wachsen, bilden die dorsale Wand der Höhle, während die eigentlichen Entodermzellen an die ventrale Wand und an die Seiten der Höhle zu liegen kommen. Zugleich wächst der dorsale Umschlagsrand nach hinten, und Hand in Hand damit wird der ursprünglich weite nach hinten offene Gastrulamund allmählich geschlossen. Dadurch kommt eine radial-unsymmetrische, aber zugleich, da die Rückenseite markiert ist, bilateral-symmetrische Gastrula zu Stande, die keineswegs als Archigastrula zu bezeichnen ist."<sup>1</sup>

We have shown that Lwoff's statement in regard to the presence of certain cells free from yolk in the dorsal wall of the archenteron is correct. The more recent writers, Klaatsch, Sobotta, and MacBride, have entirely overlooked this important point, and have needlessly criticised Lwoff in consequence. Whether those cells that form the dorsal wall of the archenteron are to be called ectoderm or endoderm is entirely, it seems to us, a matter of choice or definition.

It is the old problem of what we shall define as a germ layer — whether the presence of yolk in certain cells is, in itself, a sufficient criterion to distinguish the endoderm, or whether all the cells that are invaginated are, irrespective of their form, to be called endoderm. For ourselves, the question seems to be a trivial one, and simply as a matter of personal preference we

<sup>1</sup> Lwoff, p. 9.

choose to speak of all the cells that turn in during gastrulation as endoderm.

While we agree with Lwoff that some of the cells that are at first invaginated contain only a small amount of yolk, and clearly resemble the ectodermal cells of the dorsal side, we have found no evidence that the ectoderm continues to turn in at this point during the later period of the closing of the blastopore.

Lwoff, while admitting that Hatschek's figures are true to nature, yet disagrees with Hatschek in regard to the relation of the egg axis to that of the embryo: "Ich habe gefunden, dass der Gastrulamund von allen Seiten geschlossen wird, indem seine Ränder einander entgegenwachsen. Die Schliessung des Gastrulamundes vollzieht sich zwar ungleichmässig, aber ich habe schon gezeigt, dass die Gleichmässigkeit der Einstülpung und der Gastrulaschliessung durch die Einstülpung der Ektodermzellen am dorsalen Umschlagsrande gestört wird, indem, wie oben erwähnt, der dorsale Umschlagsrand während der Gastrulaschliessung nach hinten wächst und mehr als der ventrale und die seitlichen Ränder daran Antheil nimmt. Wenn ich das Wachsthum des dorsalen Umschlagsrandes nach hinten berücksichtige, so könnte ich auch sagen, dass die Schliessung des Gastrulamundes vorzugsweise von vorn nach hinten sich vollzieht, aber nicht in dem Sinne, wie es Hatschek will. Denn ich habe gefunden, dass die Rückenseite der Gastrula selbst nach hinten wächst, dadurch allmählig länger wird und den Gastrulamund schliesst. Während die übrigen Ränder des Gastrulamundes gleichzeitig sich zusammenziehen, wird derselbe immer kleiner. Ich habe dabei keine Spuren der Verwachsung der seitlichen Ränder von vorn nach hinten in der Medianlinie des Rückens (etwa in der Gestalt einer Nathlinie) weder an ganzen Larven noch auf den Schnitten sehen können. Indessen würde die Behauptung, dass der hinterste Theil des Gastrulamundes zuletzt übrigbleibe, nur dann reelle Bedeutung haben, wenn es nachgewiesen wäre, dass die seitlichen Ränder des Urmundes in der Medianlinie in einer von vorn nach hinten fortschreitenden Richtung verwachsen. Diese Behauptung ruht auch auf der Annahme, dass der hintere (ventrale) Rand des



Gastrulamundes während der Gastrulaschliessung unverändert bleibt. Dies ist aber auch nicht der Fall, und Hatschek's eigene Abbildungen sprechen nicht zu Gunsten dieser Annahme."<sup>1</sup>

Our view of the method of closing of the blastopore agrees essentially with Lwoff's, that is, that the blastopore closes equally from all sides, and that the gastrula axis corresponds more or less exactly with the longitudinal axis of the embryo. On the other hand, we have tried to show that there is no necessity for supposing that the cells outside the blastopore on the dorsal side turn in during the period of closure of the blastopore. It seems more probable that the advance of the dorsal lip takes place in the same way as that of the lateral and ventral lips, and even Lwoff does not suppose the latter to advance as the result of the inturning of cells.

Wilson made the important observation that "the cleavage pore, which marks the lower pole of the blastula, sometimes persists up to a stage as late as the gastrula shown in Hatschek's Figs. 26 and 27. In all such cases I examined, it lay exactly at the central point of the dome—a fact that shows that the invagination is primarily symmetrical, as originally described by Kowalevski."

Wilson also states, in contradiction to Lwoff's statement, that the entoblastic cells (macromeres) "show numerous conspicuous mitoses, and in every part of the entoblastic plate."<sup>2</sup>

Klaatsch has given a few figures, mainly optical sections of preserved embryos of *Amphioxus*. He looked for concrescence but failed to find any evidence of it. In regard to the orientation of the embryo he found the closure of the blastopore as described by Kowalevski, "von vorn herein fast genauer aboral und ganz geringe Neigung zur dorsalen Seite hin."<sup>3</sup>

Sobotta's account follows Hatschek's very closely, and adds little that is new. He states that no distinction exists between the dorsal and ventral wall of the archenteron, during the early gastrula stages, as Lwoff maintained. The explanation of this lies, no doubt, in the preserving fluids that were used. Sobotta states that in the eggs from Naples the blastopore

<sup>1</sup> Lwoff, p. 14.

<sup>2</sup> Wilson, p. 586.

<sup>3</sup> Klaatsch, p. 229.

closes later than in the Messina form, as described by Hatschek. We have had material from both localities and can state that the difference was the result of less normal development in the Neapolitan form. Sobotta has been unable to decide how the axis of the gastrula is related to the axis of the embryo. He believes that the blastopore closes equally from all points and that no concrescence takes place.

MacBride<sup>1</sup> and Sobotta have entirely overlooked the presence of smaller and lighter cells over the dorsal wall of the archenteron, and in consequence MacBride says that "it is difficult to find words to adequately characterize the artificiality and arbitrariness of such a view."

MacBride continues: "If we examine a transverse section of a completed gastrula, . . . we find no difference in character between the cells forming the dorsal wall of the alimentary canal and those forming the ventral wall, such as we should have the right to expect did Lwoff's hypothesis in any way correspond with the facts." This statement is unquestionably wrong, as our figures show.

MacBride gives an inadequate account of the process of gastrulation: "Thus I regard the gastrulation as a fairly uniform pushing in of the under or flattened surface of the blastula, accompanied by division and multiplication of the cells, such multiplication being at first most active in the dorsal (future anterior) lip of the blastopore. The blastopore, which is still wide, becomes rapidly narrowed by the upgrowth of the ventral lip; in contradistinction to what Hatschek asserts, the dorsal lip remains relatively stationary."<sup>2</sup>

No evidence is offered in support of this opinion, which is, as we have tried to show, incorrect.<sup>3</sup>

It is not our intention to enter into a comparison of the gastrulation of *Amphioxus* and of the other Chordata. The resemblance of the early larva of the Ascidians to that of *Amphioxus* is, however, so close, that a few words seem justified on this topic. The recent paper by Castle on "The Early Development

<sup>1</sup> MacBride, p. 597.

<sup>2</sup> *Ibid.*, p. 591.

<sup>3</sup> MacBride's Fig. 10 shows the endoderm of the dorsal lip of the blastopore continuing into the ectoderm on the *outer* side of the nerve tube.



of *Ciona intestinalis* " is much more detailed than the work of previous writers and we shall confine ourselves entirely to Castle's account.

In *Ciona*, at the time when the endodermal plate is bending in to form the archenteron (Castle, Fig. 78), a few cells at the dorsal lip of the blastopore also sink in and, in later stages, form the dorsal wall of the archenteron (Fig. 98). From these cells the notochord subsequently develops. They would seem, therefore, to correspond to the cells of the dorsal wall of *Amphioxus*. Outside of the semicircle of notochordal cells (Fig. 62), at the dorsal lip of the early gastrula, lie the ectodermal cells that subsequently form the nerve plate. As the blastopore closes, these cells are carried backward with the advance of the dorsal lip, but none of the cells turn in with the notochordal cells. During the backward growth of the ectodermal and notochordal cells, these cells increase in number. The nervous system of *Ciona* is derived from cells that lie at first in front of the dorsal lip of the blastopore. The nervous system of *Amphioxus* is also derived from cells in front of the dorsal lip of the blastopore, but in *Ciona* the blastopore closes from before backward, while in *Amphioxus*, if our view be correct, the closure is not from before backward, but equally from all points of the periphery. It is important to note that in *Ciona*, in which the advance of the dorsal lip is definitely shown to exist, the ectodermal cells that lie at the free edge of the dorsal lip *do not turn in during the period of closure*.

Castle derives the mesoderm of the tail from a number of cells around the posterior half of the blastopore. These cells are turned into the archenteric space during the period of gastrulation. Castle has followed the lineage of these cells and has shown that they correspond in origin with the ectodermal cells of the anterior region that form the nervous system. He therefore considers them ectodermal in origin, and consequently derives a large part of the mesoderm from the ectoderm. The more anterior mesoderm of the trunk comes from cells lying just within this semicircle of mes-ectodermal cells. This inner circle is described as endodermal in origin. Hence, in *Ciona*,

the mesoderm has a double origin — the duality, however, being a matter of definition. In *Amphioxus* there is nothing indicating that the mesoderm is derived from more than a single source.

In *Ciona* the blastopore closes on the dorsal side, and the longitudinal axis of the embryo seems to be at right angles to the primary or gastrula axis. This would be the case if the dorsal lip grew posteriorly, without the embryo changing shape. On the other hand, there are facts in the development that make it possible to interpret this backward growth of the dorsal lip as the result of a change in the shape of the entire embryo. The gastrula axis, in such a case, would shift during the closure of the blastopore. The shifting would take place in such a way that the dorsal side of the axis is carried backward and the ventral forward. The result would be that the anterior end of *Ciona* would agree with the anterior end of *Amphioxus*. We offer this only as a suggestion, for by this means the orientation of *Amphioxus* and of *Ciona* would be made to agree.

BRYN MAWR COLLEGE, BRYN MAWR, PA.,  
May 29, 1898.

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<i>a.</i>	anterior.	<i>n.</i>	notochord.
<i>b.c.</i>	body cavity.	<i>op. ect.</i>	opening in ectoderm.
<i>bp.</i>	blastopore.	<i>p.</i>	posterior.
<i>d.</i>	dorsal.	<i>s.c.</i>	segmentation cavity.
<i>ect.</i>	ectoderm.	<i>v.</i>	ventral.
<i>end.</i>	endoderm.	<i>v.p.</i>	vegetative pore.

## EXPLANATION OF PLATE XXXIII.

Figs. 1-10, except Figs. 4 *A* and 7 *A*, were drawn with Zeiss 4, oil immersion 2 mm. Figs. 4 *A* and 7 *A* were drawn with Zeiss 4 *D*. The figures in the plate were reduced one-third.

The material from which the figures (except Fig. 6) were drawn was killed in Flemming's solution, the stronger formula. Fig. 6 was drawn from material killed in Hermann's fluid. The section from which Figs. 7 *A*, *B*, *C*, *D*, and Figs. 9 and 10 were drawn was stained in iron haematoxylin. The remaining figures were drawn from unstained material.

FIG. 1. Sagittal section of an embryo at midnight. There is an artificial rupture in the ectoderm; *d.*, dorsal; *v.*, ventral.

FIG. 2. Sagittal section of an embryo at 2 A.M.

FIG. 3. Sagittal section of an embryo at 4 A.M.

FIG. 4 *A*. Oblique section of an embryo at 5 A.M.

FIG. 4 *B*. Enlarged drawing of area marked *x* in 4 *A*.

FIG. 5. Sagittal section of an embryo at 6 A.M.

FIG. 6. Part of a cross-section through the posterior third of an embryo at 8 A.M.

FIG. 7 *A*. Sagittal section through an embryo at 11 A.M.

FIG. 7 *B*. Enlarged drawing of area marked *x* in 7 *A*.

FIG. 7 *C*. Enlarged drawing of area marked *y* in 7 *A*.

FIG. 7 *D*. Enlarged drawing of area marked *z* in 7 *A*.

FIG. 8. Drawn from the anterior region of a longitudinal section of an embryo with eight body cavities; *ect.*, ectoderm; *end.*, endoderm; *b.c.*, body cavity.

FIG. 9. From the anterior wall of an embryo, showing a portion of a cell after division.

FIG. 10. Dividing cell from the endoderm of Fig. 16.













## EXPLANATION OF PLATE XXXIV.

Figs. 11-16, and 19, 20, were drawn with Zeiss 4, oil immersion 2 mm. Figs. 17 and 18 were drawn with Zeiss 4 D. The figures on the plate were reduced one-third.

The embryo from which Fig. 11 was drawn was killed in Flemming's solution and was unstained. Figs. 16-18 were drawn from material killed in Flemming's solution, the stronger formula, and stained in iron haematoxylin. The remaining figures were drawn from material killed in corrosive acetic and stained with lithium carmine.

FIG. 11. Blastopore drawn from a total mount at about 2 A.M.

FIG. 12. Blastopore from a total mount at about 3 A.M., showing also endoderm cells through the blastopore opening.

FIG. 13. Blastopore from total mount at about 5 A.M.

FIG. 14. Blastopore from total mount at about 6 A.M.

FIG. 15. Optical section through an embryo at 2 A.M.

FIG. 16. Embryo with endoderm cells turned outward around the vegetative pore.

FIG. 17. Embryo with vegetative pore.

FIG. 18. Embryo with opening at animal pole.

FIG. 19. Embryo, showing vegetative pore at 6 A.M.

FIG. 20. Oblique section of an embryo at 4.30 A.M., showing cells during and after division (at *x*).

Fig 11

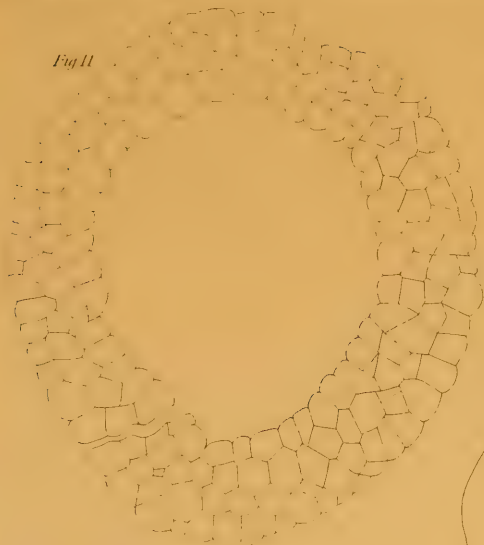


Fig 12



Fig 13

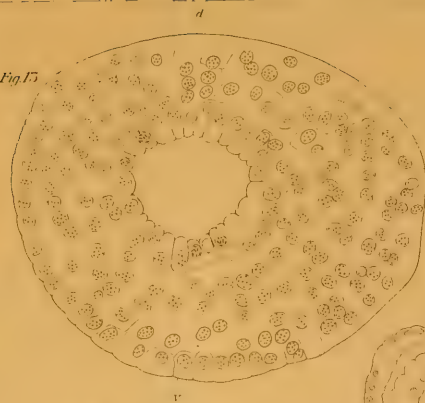


Fig 14

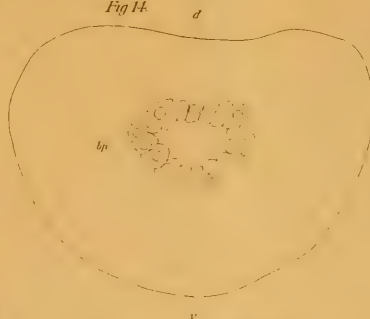


Fig 20



Fig 19



Fig 18  
spiral



Fig 17

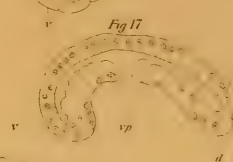


Fig 16

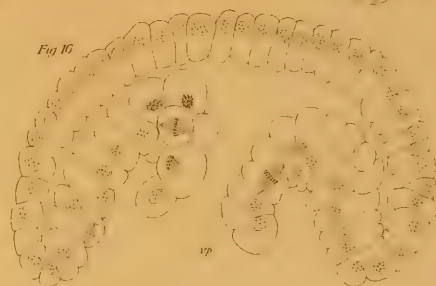
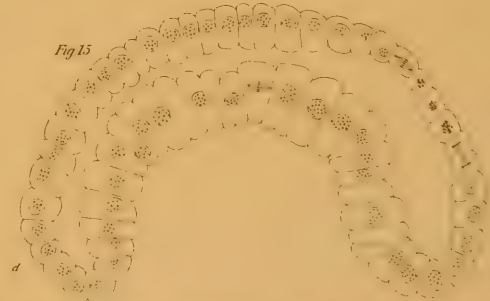


Fig 15







# PHOTOGRAPHS OF THE EGG OF ALLOLOBOPHORA FOETIDA.

KATHARINE FOOT AND ELLA CHURCH STROBELL.

## I.

THIS paper is the first of a series in which we hope to make a careful comparative study—illustrated with photographs—of the effects of various fixatives on the cytoplasm of the egg of *Allolobophora foetida*.<sup>1</sup>

In addition a number of preparations have been photographed to illustrate the following points:

The morphological resemblance of the fertilization cone to the male aster.

The position of the middle-piece in the male aster.

The origin of the sperm granules.

The early stages of the development of the pronuclei.

The presence of osmophile granules in the nucleoli of the germinal vesicles.

The photographs have been taken at only two magnifications (660 and 950). No photographic feats have been attempted, photography being used merely to register the points we wish to illustrate, and the higher magnification has not been used where our aim could be achieved by the lower.

### *Cytoplasmic Reaction to Fixatives.*

Our aim is to make a comparative study of the effect produced not only by the compound fixatives, but by the component parts of each. If with a given fixative we find a definite cytoplasmic configuration, can this be materially changed by omitting one of the constituents of the fixative? If this

<sup>1</sup> A few photographic illustrations of this comparative work were published in 1898. Foot and Strobell, "Further Notes on the Egg of *Allolobophora foetida*," *Zoölogical Bulletin*, vol. ii, No. 3.

proves to be the case, will this constituent produce a like effect in combination with another fixative? We hope by a careful comparative study to be able to determine how much of the structure seen in fixed cytoplasm is due to the fixation.

The cytoplasm reacts very differently to different fixatives; for example, the spaces occupied by the hyalin globules<sup>1</sup> are in some cases distinctly defined (photo. 17), while in others the globules have apparently fused in all directions, producing a scattering of the intermediary substance and consequent formation of rays.

It is a significant fact that in those preparations where the spherical spaces occupied by the hyalin globules are destroyed, we usually find more definite rays, this being clearly shown by comparing photo. 17 with photos. 15, 19, 21, and 22 (these five photos. showing nearly the same stage of development of the egg).

The last four, with many of the chromo-acetic preparations (*e.g.*, 2, 8 and 9), might be called in evidence for the reticular theory of cytoplasm, and photo. 17 supports with equal force the alveolar theory.

We are convinced that the indication of rays we have seen in the *living* eggs is not comparable to the rays seen in preparations where the hyalin globules have fused and scattered.

Twelve fixatives are represented in our three plates, but we do not wish to assert that any one of these preparations represents the typical effect of the special fixative, the reaction of the egg to any one fixative being very inconstant. This complicates the problem and a comparative study can be profitably carried out only by an exhaustive collection of photographs. In the present paper attention will be called to a few suggestive comparisons, awaiting further data before attempting to draw definite conclusions.

<sup>1</sup> At Dr. Whitman's suggestion we have adopted the term hyalin globules to designate the substance which in an earlier paper we called sap globules. This substance, which is in globular form only at certain stages, we interpret as synonymous with the hyaloplasm of some authors. We retain the term globule, because when this substance is pressed out of the living egg, it keeps its globular form, not fusing with water for several minutes. Treatment with osmic acid has failed to demonstrate any fatty constituent.











*Archoplasm in the Male Attraction Sphere.*<sup>1</sup>

Sometimes the archoplasm is quite uniformly distributed, and again it is represented by rods or rays or granular masses. As these varying forms are seen at the same stage of development of the egg, is it not probable that they represent varying expressions of the effect of the fixatives?

An examination of the photos. of eggs killed in corrosive-sublimate (12, 14, 15, 18, 20) reveals a marked similarity in the distribution of the archoplasm. It forms a granular, flocky circle around the male attraction sphere, and this arrangement is relatively constant for corrosive-sublimate preparations.

A comparison of these with photo. 17 shows a striking difference, and we are forced to conclude that the approximately even distribution of archoplasm in this section is more suggestive of the living condition, because the hyalin globules have not fused, as is probably the case in the corrosive-sublimate preparations, and the action of the fixative must be thus less injurious. In the above list of corrosive-sublimate preparations we did not include photo. 16, for the arrangement of the archoplasm of that section is exceptional—it is more like that shown in photo. 17, and it is a significant fact that the fixation of the rest of the cytoplasm is also more like that of photo. 17, *i.e.*, there has been less fusing of the hyalin globules. The egg of photo. 17 was fixed with Hermann's fluid, *without* acetic acid; the same stage fixed with Hermann, in which the acetic acid had been retained, shows a very different cytoplasmic configuration.

The archoplasm of photo. 21 (Perenyi's fluid) and of 19 (Flemming's fluid strong) is aggregated into decided rays, and there are no indications of the presence of hyalin globules.

The archoplasm of photo. 7 is more condensed than is the case in any of the other preparations. The fixative used (osmic

<sup>1</sup> We are preparing a paper to demonstrate (with a series of photographs) the presence of the archoplasm throughout the cytoplasm and its homology to the so-called yolk-nucleus. We hope to defend this broader use of the term, employed in an earlier paper, and to support the interpretations there suggested. Foot, "Yolk-Nucleus and Polar Rings," *Journ. of Morph.*, vol. xii, No. 1, 1896.

and acetic) shrunk the egg nearly one-half its diameter, and the archoplasm is shrunken into compact masses that resemble rods.

The archoplasm of photo. 9 (chromo-acetic) forms a sharp contrast to that of photo. 7. Part of it is aggregated at the center of the attraction sphere, where it is stained so deeply that the middle-piece is completely obliterated.

#### *Fertilization Cone.*

Photo. 1 shows the size of the cone in relation to the entire egg (at this stage the first maturation spindle is in the metaphase and at the periphery of the egg). Photo. 2 is a section of such an egg, cut longitudinally through the cone. The thinness of the section enables us to see the head of the sperm within the cone, on its way to the center of the egg.<sup>1</sup> The sperm continues its course until its middle-piece is near the inner aster of the first maturation spindle, slightly turning and bringing its head again near the periphery, some of the preparations (for example, photo. 12) producing the false impression that it has entered from the latter point, and the aster has formed at the apex of the head. The progress of the sperm towards the inner aster of the spindle appears to be dependent upon the stage of development of the egg. When the spindle has reached the anaphase, the sperm aster is formed and the progress of the sperm towards the center of the egg then ceases, the head separates from the middle-piece and contracts into a short thick rod (photos. 8, 16, 17, and 18). The spiral twist of the sperm shown in photo. 2 is an uncommon form, but it probably does not indicate an abnormal condition of the egg.

In photo. 5 we have an unmistakably pathological cone, this enabling us to determine the pathological condition of the rest of the cytoplasm and giving a standard of comparison which may prove of service.

Photo. 6 probably represents another example of pathological cytoplasm, as the egg contains four spermatozoa, two of which are shown in the section photographed. It is a question

<sup>1</sup> Over-printing at the apex of the cone has obliterated the sperm at that point.

how much of the difference in the cytoplasmic configuration of photos. 5 and 6 is due to the difference in fixation, the egg of photo. 5 having been killed in corrosive-sublimite and that of photo. 6 in picro-formalin. Photo. 6 represents, however, a little later stage of development of the egg.

*Morphological Similarity of the Cone and Male Aster.*

It is impossible to avoid drawing conclusions as to the morphological significance of the resemblance between the male aster and transverse sections through the fertilization cone.

Photo. 3 shows a transverse section of a fertilization cone, near its apex, and a comparison of this with a section through the male aster of photo. 9 will serve to illustrate this point. The rays and the central aggregation of archoplasm are as pronounced in the one as in the other, suggesting that each end of the head of the sperm — the spine and the middle-piece (see photo. 37) produces on the cytoplasm of the egg a like morphological effect. This would indicate that the spine and the middle-piece are of the same substance, though the identity cannot be complete, as the cytoplasm does not react to the two structures at the same stage of development of the egg. There are a few investigators who claim to have traced a substance in the spermatid to both spine and middle-piece. The effect produced by the spine is made, however, by a *moving* object (the sperm entering the egg), and we have thus a different-shaped "aster" — a cone-shaped aster. Is it possible that this may have any bearing on the opposing interpretations of various authors, some asserting that the anterior end of the head of the sperm produces the male aster, and others that the posterior end of the head (the middle-piece) produces it?

If we accept the interpretation of those authors who claim to have traced a part of the aster of the spermatid to *both* spine and middle-piece, may we not regard that part of the spermatozoön (including spine, head, and middle-piece) as an attenuated spindle,<sup>1</sup> and expect that each end of this spindle

<sup>1</sup> Foot, "The Centrosomes of the Fertilized Egg of *Allolobophora foetida*," *Biol. Lect., Marine Biological Laboratory.* Boston, 1896.

will produce a like morphological effect upon the cytoplasm of the egg? We have been unable to differentiate in either spine or middle-piece any special structure that we feel justified in interpreting as a centrosome.

*Further Observations on the Middle-Piece of the Sperm and its Morphological Rôle in the Male Aster.*

In 1897<sup>1</sup> one of us differentiated, in color the centrosome of the male aster from the middle-piece of the spermatozoon, this leading to the interpretation that the centrosome of the male aster is of purely cytoplasmic origin — the middle-piece merely producing the cytoplasmic phenomenon known as the aster, but no definite part of the sphere being formed of the middle-piece substance.

Further investigation has demonstrated that the middle-piece can remain for a definite period intact within the aster, and that the later differentiation in color is probably due to chemical change, for it disintegrates before disappearing.

The photos. of Pl. XXXVI show the middle-piece within the aster. Although it is by no means always in the center, we have been able to find no other structure that we feel justified in interpreting as a centrosome.

The middle-piece finally disintegrates and totally disappears, and there is no evidence that it takes any part in forming the cleavage centrosomes. As stated in the paper above referred to (I), during the formation of the young pronuclei both the egg and sperm centrosomes totally disappear, and there is no evidence that either takes part in forming the cleavage centrosomes, this egg supporting the theory that the cleavage centrosomes arise *de novo* in the cytoplasm.

In photo. 12 we see a portion of the head of the spermatozoon, its middle-piece within the aster, and a part of the tail (the missing part of the head is in the next section). The head at this stage shows constrictions at definite intervals (these are lost in some of the reproductions of photo. 12, but

<sup>1</sup> Foot, "The Origin of the Cleavage Centrosomes," *Journ. of Morph.*, vol. xii, No. 3, 1897.



two are shown in photo. 14). We are unable at present to homologize these divisions of the sperm head with the chromosomes of the egg, not having seen the requisite number.

If the archoplasm shown at the periphery of this sphere is comparable to Boveri's archoplasm in ascaris, it certainly is not brought in by the sperm, as claimed by several authors for the ascaris egg, for here we see the spermatozoön still intact.

Photo. 13 shows part of the head of the sperm and the entire middle-piece, at about the same stage as that of photo. 12, though the magnification is somewhat greater (950). Without more data we hesitate to interpret the two tiny filaments at the posterior end of the middle-piece. One of them may be the proximal end of the tail of the spermatozoön, or they may both represent the splitting of the proximal part of the tail and its fusing with the cytoplasm of the egg. This egg was killed in 2 per cent osmic in 70° alcohol, and it is interesting to compare its cytoplasmic structure with that of photo. 12, in which case the egg was killed in corrosive-sublimate.

In photo. 14 we have the same stage of development of both sperm and middle-piece.

In photo. 15 we have transverse sections of two middle-pieces within the aster, and it is scarcely necessary to call attention to their resemblance to a dividing centrosome. The heads of the two spermatozoa are in the adjacent sections. They are contracted rods such as those shown in photos. 16, 17, and 18.

Photo. 16 shows the head of the spermatozoön separated from the middle-piece and contracted into a relatively thick short rod — the middle-piece has (apparently) contracted somewhat, and we interpret the filament to the right of the middle-piece as a part of the tail of the spermatozoön.

In the preparation represented by photo. 17 there is a middle-piece distinctly seen within the sphere; but it has been obliterated by too dark printing in the reproduction, over-printing producing the same results as over-staining.

In photo. 18 the middle-piece is by no means in the center of the sphere; but the spherical form of the latter remains intact. If the middle-piece does more than stimulate the egg

to the expression of cell activity known as the male attraction sphere, if it is the organic center of the sphere, forming with the rays an organic connected whole, would not the spherical form of the sphere be disturbed by the middle-piece moving away from the center? In reality the form is no more disturbed than is the form of the fertilization cone, when we find the head of the spermatozoön quite out of the center of the cone.

In photo. 19 the middle-piece is in nearly the center of the sphere. (The contracted head of the sperm is in the adjacent sections.)

In photo. 20 we have a transverse cut through the middle-piece, which bears a marked resemblance to a centrosome, though its position in the sphere is eccentric; the spherical form of the sphere, however, remains undisturbed. (The contracted head of the sperm is in the next section.)

Photo. 21 shows a little later stage of development than that of 18; the middle-piece is beginning to disintegrate, and at a still later stage it has entirely disappeared. The differentiation in color which one of us obtained between the middle-piece of the spermatozoön and a distinct spherical body within the male aster was probably due to the chemical change which must take place at this time of disintegration.

Photo. 22 shows about the same stage of development as that of photo. 21; the middle-piece is beginning to disintegrate, showing three tiny spherical bodies. The middle-piece and rays finally disappear at about the same time.

#### *Further Observations on the Origin of the Sperm Granules.*

Photos. 10 and 11 indicate an origin of the pathological sperm granules different from that suggested by one of us in 1897.<sup>1</sup>

They were interpreted then as being formed at the expense of the archoplasm, for the reason that in those cases where they were present in the cone they were surrounded by an area free from archoplasm, indicating that they arose by a concentration of the archoplasm substance at that point.

<sup>1</sup> Foot, "The Origin of the Cleavage Centrosome," *Journ. of Morph.*, vol. xii, No. 3, 1897.



We should expect to find them formed at the expense of some substance in the cell, as they are not constant structures and appear to be a pathological feature. In photo. 10 we have an exaggerated expression of the phenomenon (an unusual number of granules), this aiding us in interpreting their origin. This photograph indicates that they have arisen at the expense of the head of the sperm itself. Less than half of the length of the head is shown in this section; but a comparison of its diameter with that shown in photo. 2 indicates a great loss of substance.

In photo. 11 we see a part of a contracted head of a sperm which appears to have been fixed in the act of constricting off a sperm granule.<sup>1</sup>

#### *Early Stages of Development of the Pronuclei.*

The photographs of Plate XXXVII illustrate the early stages of development of the male and female pronuclei — four fixatives being represented.

Photo. 23 shows a transverse view through the second maturation spindle, which has reached the anaphase of development. These are the chromosomes approaching the inner pole of the spindle, and which are destined to form the female pronucleus. There are eleven chromosomes in the first and second spindles, and in this photo. each one of the eleven is shown. This photo. was taken by Dr. Fuller, from a slightly crushed oöcyte second order.

Photo. 24 shows a little later stage of development (telophase). This section contains five or six of the chromosomes,

<sup>1</sup> In the *Journ. of Morph.*, vol. xvi, No. 1, 1899, Byrnes describes small round bodies often accompanying the sperm-nucleus in *Limax agrestis*, and says they may owe their origin — although she does not illustrate this point — to particles of chromatin constricted off from the sperm-nucleus before it becomes vesicular, having seen a "few cases in which a portion of the chromatin seemed to be in process of constricting from the sperm head." As she does not suggest they are a pathological expression, on the contrary implying they have a function, it is a question whether they are the same structures we show in photos. 11 and 12. We regret the necessity of mentioning Miss Byrnes's paper in a footnote. The number of the journal in which it appeared was issued in June, 1900, and her paper was not read until just before the receipt of our final proofs.

which have reached the inner pole of the second spindle, the rays of the aster still persisting. A comparison with photo. 23 indicates that these ring chromosomes have been formed by the uniting of the free ends of *V*-shaped chromosomes, such as those shown in photo. 23.

Photo. 25 shows two (of the eleven) chromosomes at a little later stage of development. Besides the ring, a tiny spherical body appears in connection with each chromosome, and we interpret these as the first appearance of the nucleoli of the female pronucleus, the periphery of each ring representing the chromatin of the chromosomes.<sup>1</sup> If this interpretation is correct, then the periphery of the vesicles which characterize the *later* stages must be interpreted as chromatin, and the spherical body in connection with each, as nucleolar substance. These later stages are shown in photo. 26 (where three of the eleven vesicles are represented) and in photos. 27, 28, 29, 30, 31, and 32. In photo. 27 one of the vesicles shows thick threads of chromatin connecting the nucleolus with the periphery of the vesicle.

Photo. 33 shows part of the head of the spermatozoön breaking up into similar vesicles—the periphery of the vesicle upon which we have focussed we interpret as of chromatin, and the tiny spherical body as nucleolus.

Photo. 34 shows a later stage of the development of the spermatozoön into the male pronucleus, several of the vesicles having fused into one, the rest being in adjacent sections.

### *Osmophile Granules in the Nucleoli.*

It is exceptional to find osmophile granules in the nucleoli, and we are therefore inclined to regard them as abnormal features. A nucleolus from a germinal vesicle of an unstained ovarian egg is shown in photo. 35. The ovary was fixed in chromo-acetic, washed in water and immersed for one hour in osmic in order to blacken the dentoplasmic granules.

<sup>1</sup> In these vesicles, at a little later stage of development, one of us differentiated in color the nucleolus from the peripheral chromatin ring. Foot, "The Origin of the Cleavage Centrosomes," *Journ. of Morph.*, vol. xii, No. 3, 1897.

After photographing, this ovary was immersed for fifty-three hours in turpentine and the same nucleolus again photographed without staining (photo. 36). The fat granules (osmophile granules) had neither dissolved out nor faded in any part of the ovary. This discredits the advice of those authors who recommend placing sections for twenty-four hours in turpentine or xylol to remove the fat granules before staining. In some cases, perhaps as a rule, warm xylol or turpentine will fade the blackening caused by osmic, but it is by no means infallible, and, moreover, the xylol very often does not dissolve the fat substance. Photographs have enabled us to discover its presence after a careful examination under the microscope had convinced us it had dissolved out completely. Again, after long immersion in turpentine, and after staining, the granules are still sharply blackened, and confidence in the certainty of their removal by turpentine leads one to misinterpret them as other than fat granules.

Further details regarding the nucleoli and the ovarian egg will be discussed and illustrated by photographs in a paper now being prepared for press.

#### *Method.*

Further experiments with the mechanical mode of focussing described in our last paper<sup>1</sup> have led to the development of a simpler and more rapid method of overcoming one of the practical difficulties encountered by the cytologist in photography.

After abandoning the effort to focus fine details on the ground glass of the camera, or through a transparent portion marked off in the ground glass, we used the method described in detail in the paper just referred to—ascertaining by experiment with a large object, easily focussed on the ground glass (a sharply stained nucleolus, for example), just what difference the pointer on the micrometer screw registered, between the focus through the microscope and the focus on the ground

<sup>1</sup> "Further Notes on the Egg of *Allolobophora foetida*," *Zoölogical Bulletin*, vol. ii, No. 3, 1899.

glass. This difference we found to be  $\frac{3}{20}$  of one of the twenty-five divisions marked on face of micrometer screw.<sup>1</sup>

In the practical use of this method, the suggestion for increasing its accuracy came through observing the variation in the turn required by the micrometer screw, dependent upon the operator's eyeglasses, whether reading or distance glasses were worn. This suggested that there must be a lens which, when once adapted to the operator's eyes, would give with unflinching accuracy the plane required for the focus on the ground glass. A series of spherical lenses, from -1.D. to -5.D., were tested in the following manner:

A number of small microsomes were carefully focussed through the microscope, with projection ocular IV (diaphragm at 0), the operator wearing ordinary distance spectacles, or in a case where the sight was normal, no glasses were used. The micrometer screw was then turned to raise the focus the number of points found by former tests, to give the camera focus. By this change of screw the microsomes originally focussed upon were of course completely lost sight of. Leaving the screw at this point, the spherical (minus) lenses were tested, beginning with the lowest number, placing them one at a time on the projection ocular, until one was found which brought the microsomes in sight and gave the desired focus, exact in every detail. In making this test, if the camera focus has not been obtained mechanically (*i.e.*, the difference in the two foci measured by points on micrometer screw) the most accurate way of getting at it is to take a series of photographs, focussing through the minus lenses. Beginning with the lowest number, develop each plate as it is taken, until a negative giving the desired focus demonstrates which lens is needed. The proper lens, when found, can be mounted in ordinary spectacle frames, or simply laid on top of the projection ocular, as the operator finds most convenient.

To insure faithful results it is advisable to make a careful sketch, for reference, of a few of the most minute details, in the section to be photographed. After focussing for a

<sup>1</sup> These figures are given merely to illustrate the method, each microscope requiring a special test.











photograph, let the microscope stand undisturbed for at least ten minutes before pulling down the camera, and never attempt to take a photograph unless the focus has held absolutely true during this interval. After the photograph is taken, raise the camera and again examine the preparation to see that the focus has held while the plate was being exposed. Attention should be given to the working condition of the micrometer screw; it should be tight enough to preclude any chance of slipping after the focus is finally adjusted.

A special test must be made for each magnification, but this need not mean undue experimenting, for two magnifications should be adequate for practical use, the lower for photographing entire sections, and the higher for special details, such as centrosomes. Restricting one's photographs, as far as possible, to one magnification, offers a great advantage for comparison of shrinkages.<sup>1</sup>

We feel sure that our photographs in this paper are merely a promise of better results. It has been necessary in the reproduction to sacrifice many minor details to bring out a few important points. Our original prints are partly responsible for this, for in them, to insure a successful reproduction of the salient points in a section, we sacrificed the general cytoplasmic structure. For example, we have experimented with photo. 17, and have made a much stronger impression of the cytoplasm, and at the same time have not obliterated the middle-piece in the aster by over-printing.

<sup>1</sup> We have taken a photograph of a stage micrometer at the two magnifications we use, 660 and 950. A print from this negative gives a scale by which any detail in the photograph can be readily measured.

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#### EXPLANATION OF PLATES.

All the photographs, except Nos. 1, 8, 9, 23, 35, 36, and 37, were taken in the winter of 1899, on the Lumière (France) or Nye (Belgium) plates.

In three or four cases a detail in the original print has been slightly strengthened with a lead-pencil, merely enough to overcome in part what is lost by reproduction.

If any of our readers should wish to compare the reproductions with the solar prints, one or more may be obtained on request.

## EXPLANATION OF PLATE XXXV.

PHOTO. 1. An entire egg (oöcyte first order). This photograph was taken by Dr. Charles G. Fuller, of Chicago, in the fall of 1893. He focussed on the fertilization cone, and although the magnification is not great, the photograph appears to us as very satisfactory in showing the size of the cone in relation to the entire egg. Fixative, chromo-acetic. Stain, alum-cochineal. The reproduction of the cone in this figure has not been uniformly successful.

PHOTO. 2. Section ( $3\mu$ ) of oöcyte first order. Fertilization cone cut longitudinally. Head of the sperm within the cone, showing a spiral twist. Cytoplasm typical of chromo-acetic preparations at this stage. The osmophile granules are stained. Fixative, chromo-acetic. Stain, iron-haematoxylin.  $\times 660$ .

PHOTO. 3. Section ( $3\mu$ ) of oöcyte first order. Fertilization cone cut transversely near its apex. Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 660$ .

A comparison of photos. 2 and 3 illustrates the relative shrinkage produced by the two fixatives and the difference in the topography of the cytoplasm.

PHOTO. 4. Vignetted section ( $3\mu$ ) of oöcyte first order. Fertilization cone cut transversely about midway between its base and apex. Fixative, osmic and acetic acid. Stain, iron-haematoxylin.  $\times 660$ .

PHOTO. 5. Section ( $3\mu$ ) of a pathological oöcyte first order. Fertilization cone cut longitudinally. Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 660$ .

PHOTO. 6. Vignetted section ( $3\mu$ ) of a polysperm oöcyte first order. The fertilization cone has almost disappeared, but shows that it contained two spermatozoa. Fixative, picro-formalin. Stain, iron-haematoxylin.  $\times 660$ .

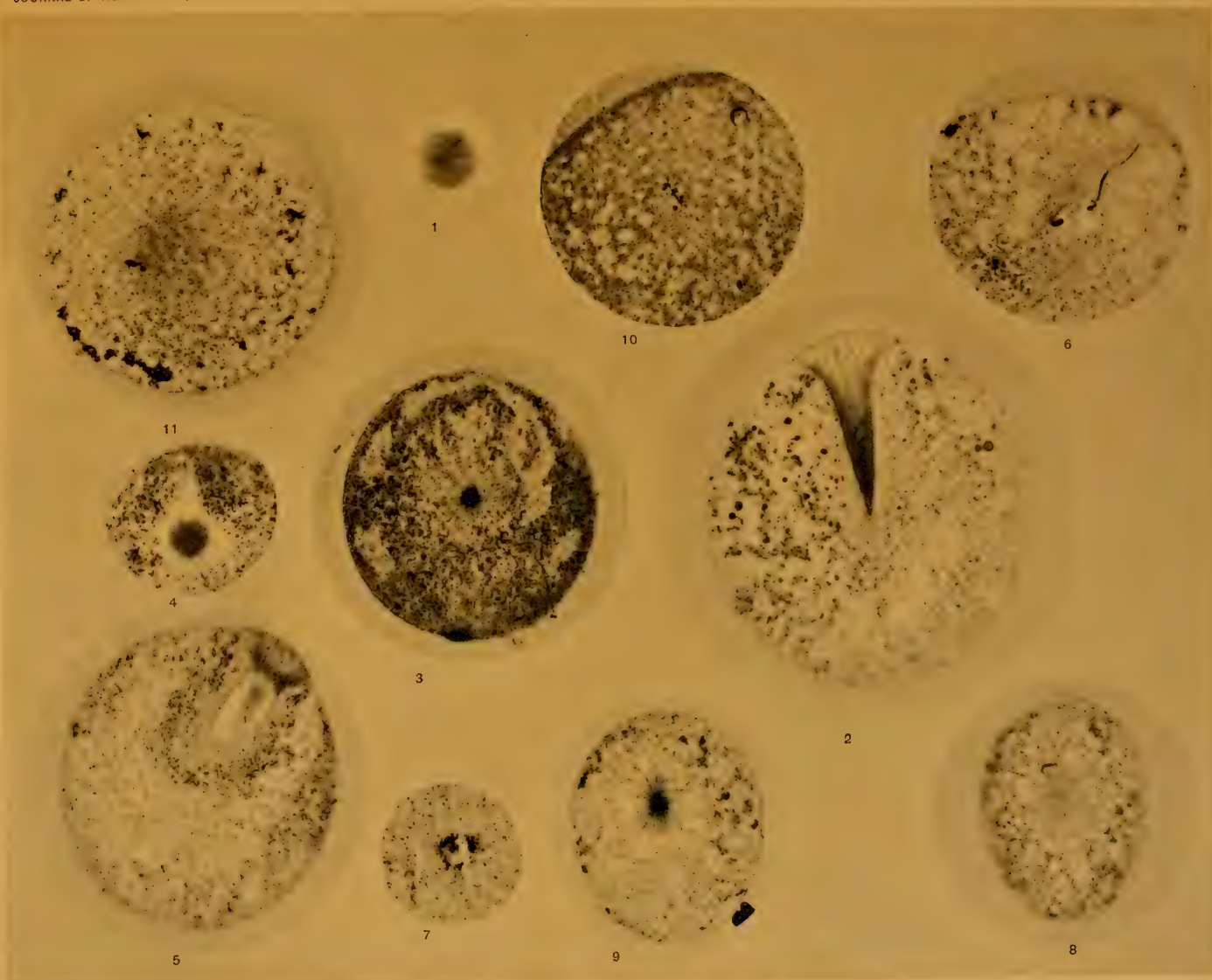
PHOTO. 7. Vignetted section ( $3\mu$ ) of oöcyte second order, showing a sperm aster, with the middle-piece of the sperm near its center. The head of the sperm appears in the next section as a rod, as shown in photos. 8, 16, 17, 18. Fixative, osmic and acetic. Stain, iron-haematoxylin.  $\times 660$ .

PHOTO. 8. Section ( $7\mu$ ) of oöcyte second order, showing a part of the sperm head contracted into a rod and an indication of the sperm aster, the section being through the periphery of the aster. This photo. was taken by Dr. Fuller, 1894. Fixative, chromo-acetic. Stain, iron-haematoxylin.  $\times$  about 400.

PHOTO. 9. Section ( $7\mu$ ) of oöcyte second order, showing male aster, the center of which is stained so deeply that the middle-piece is completely obliterated. Photographed by Dr. Fuller, 1894. Fixative, chromo-acetic. Stain, iron-haematoxylin.  $\times$  about 400.

PHOTO. 10. Vignetted section ( $3\mu$ ) of oöcyte second order, showing a part of the head of the sperm surrounded by sperm granules. Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 950$ .

PHOTO. 11. Section ( $3\mu$ ) of oöcyte second order, showing a part of a contracted head of a spermatozoön which appears to have been fixed in the act of constricting off a sperm granule. Fixative, Flemming's fluid (strong). Stain, iron-haematoxylin.  $\times 660$ .









## EXPLANATION OF PLATE XXXVI.

PHOTO. 12. Section ( $3\mu$ ) of oöcyte second order, showing the head of the spermatozoön, its middle-piece within the aster and a part of the tail. (The missing part of the head is in the next section.) Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 660$ .

PHOTO. 13. Vignetted section ( $3\mu$ ) of oöcyte second order, showing part of the head of the spermatozoön, the middle-piece, and (probably) a tiny piece of the proximal end of the tail. Stage of development about the same as that of photo. 12. Fixative, 2% osmic in  $70^\circ$  alcohol. Stain, iron-haematoxylin.  $\times 950$ .

PHOTO. 14. Vignetted section ( $3\mu$ ) of oöcyte second order. Stage of development about the same as that of photo. 13. Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 950$ .

PHOTO. 15. Vignetted section ( $3\mu$ ) of oöcyte second order. Stage of development about the same as that of photo. 14. The section has cut transversely two middle-pieces which are within the aster. (The heads of the two spermatozoa are in adjacent sections.) Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 950$ .

PHOTO. 16. Vignetted section ( $3\mu$ ) of oöcyte second order, showing the head of the spermatozoön separated from the middle-piece and contracted into a relatively thick short rod. Within the aster the middle-piece and probably a piece of the tail. Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 950$ .

PHOTO. 17. Section ( $3\mu$ ) of oöcyte second order, showing the contracted head of the spermatozoön separated from the middle-piece, which is within the sphere, but over-printing has obliterated it. The hyalin globules larger than those seen in the normal living egg at this stage. Fixative, Hermann's fluid *without* acetic acid. Stain, iron-haematoxylin.  $\times 660$ .

PHOTO. 18. Vignetted section ( $3\mu$ ) of oöcyte second order. Stage of development about the same as that of photos. 16 and 17. Middle-piece not in the center of the sphere. Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 950$ .

PHOTO. 19. Vignetted section ( $3\mu$ ) of oöcyte second order. Middle-piece nearly in the center of the sphere. The contracted head of the sperm is in the adjacent section. Fixative, Flemming's fluid (strong). Stain, iron-haematoxylin.  $\times 950$ .

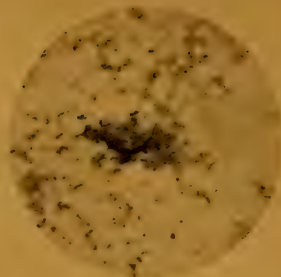
PHOTO. 20. Vignetted section ( $3\mu$ ) of oöcyte second order, showing a transverse cut through the middle-piece of the spermatozoön, its position in the sphere eccentric. The head of the spermatozoön is in the next section. Fixative, corrosive-sublimate. Stain, iron-haematoxylin.  $\times 950$ .

PHOTO. 21. Vignetted section ( $3\mu$ ) of oöcyte second order, showing a little later stage of development than that of 16-19. The middle-piece is beginning to disintegrate. The contracted head of the sperm is seen in the next section. Fixative, Perenyi's fluid. Stain, iron-haematoxylin.  $\times 950$ .

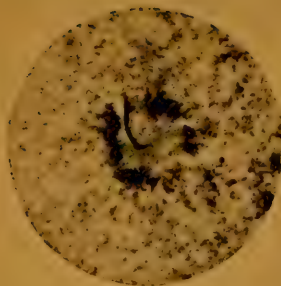
PHOTO. 22. Vignetted section ( $3\mu$ ) of oöcyte second order at about the same stage of development as that of photo. 21, the middle-piece showing like evidences of disintegration. Fixative, picro-formalin. Stain, iron-haematoxylin.  $\times 950$ .



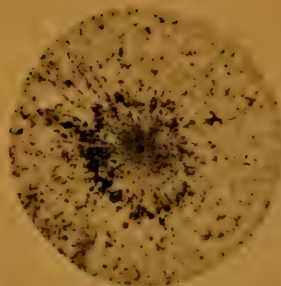
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13.



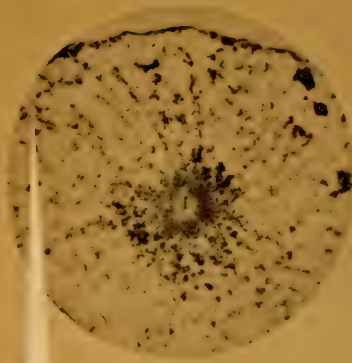
14.



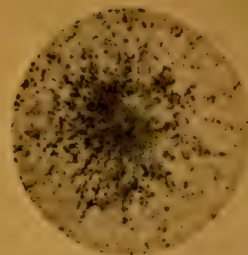
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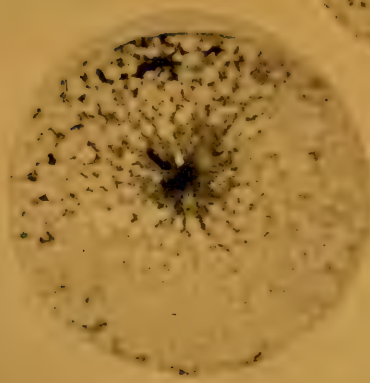
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22.



19.



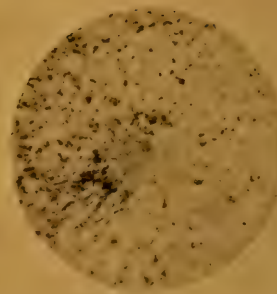
17.



18.



21.



20.





## EXPLANATION OF PLATE XXXVII.

The photos. of this plate show the early stages of development of the male and female pronuclei, four fixatives being represented.

PHOTO. 23. Shows a transverse view of the second spindle, which has reached the anaphase of development. Photo. taken by Dr. Fuller. Fixative, chromo-acetic. Stain, alum-cochineal.  $\times$  about 1000.

PHOTO. 24. Vignetted section ( $3\mu$ ) of mature egg, showing five of the eleven chromosomes which have reached the inner pole of the second spindle, the aster rays of which are still present. Fixative, 2% acetic. Stain, iron-haematoxylin.  $\times$  950.

PHOTO. 25. Section ( $3\mu$ ) of mature egg, showing a little later stage of the chromosomes than that of photo. 24. In this section only two of the eleven chromosomes are seen. Within each tiny vesicle is a minute spherical body which we interpret as the first appearance of a nucleolus. Fixative, picro-acetic. Stain, iron-haematoxylin.  $\times$  660. The reproduction of this photograph is not satisfactory. In the solar print the chromatin rings are nearly as sharp as those shown in photo. 24, and on the inner side of each ring there is a tiny sharp granule.

PHOTOS. 26-32 are vignetted sections ( $3\mu$ ) of mature eggs, showing later stages of development of the vesicles, from three to five vesicles in each section.

PHOTO. 31 shows the second polar body on the periphery.

Fixative, photos. 26 and 28, 2% acetic.  $\times$  660. Fixative, photos. 27, 29, 30, and 32, chromo-acetic.  $\times$  660. Fixative, photo. 31, Flemming's fluid (strong). Stain, iron-haematoxylin.  $\times$  660.

PHOTO. 33. Section ( $3\mu$ ) of mature egg, showing part of the head of the spermatozoön breaking up into vesicles similar to those destined for the female pronucleus, *i.e.*, photos. 24-32. Fixative, picro-acetic. Stain, iron-haematoxylin.  $\times$  660.

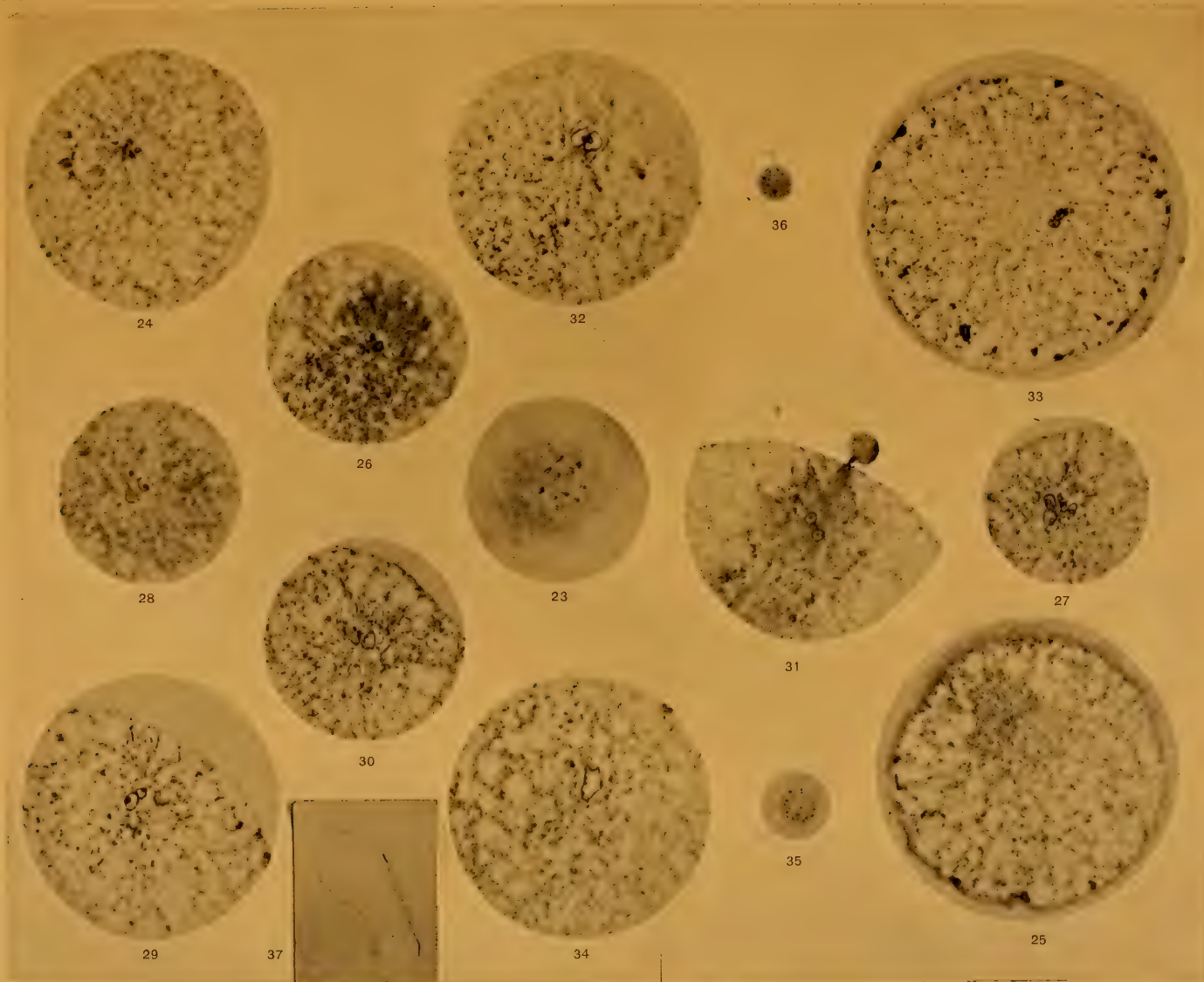
PHOTO. 34. Vignetted section ( $3\mu$ ) of mature egg, showing a later stage of development of the male pronucleus than that of photo. 33. Only a part of the pronucleus is present in this section. Fixative, chromo-acetic. Stain, iron-haematoxylin.  $\times$  660.

PHOTO. 35. A nucleolus from a germinal vesicle of an ovarian egg, showing the presence of osmophile granules in an unstained preparation. Fixative, chromo-acetic, followed by osmic acid.  $\times$  950.

PHOTO. 36. The same nucleolus (at a slightly different focus) after the sections had been immersed for fifty-three hours in turpentine. Preparation still unstained.  $\times$  950.

PHOTO. 37. Spermatozoön showing spine, head, middle-piece, and tail. This was taken from a slime tube in which the two cocoons were in process of formation. Spine and middle-piece only are stained. S





















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